Journal of Surgical Oncology 1998;68:231–236

A Cross-Sectional Study of Vitamin Intake in Postoperative Non-Small Cell Lung Cancer Patients

AMINAH JATOI, MD,[1,2]* BENEDICT D.T. DALY, MD,[3] GERTRUDE KRAMER, MD,[4] AND JOEL B. MASON, MD[1]

[1]*Division of Clinical Nutrition, Department of Medicine, Tufts University and New England Medical Center, Boston, Massachusetts*
[2]*Division of Hematology/Oncology, Department of Medicine, Tufts University and New England Medical Center, Boston, Massachusetts*
[3]*Division of Cardiothoracic Surgery, Department of Surgery, Tufts University and New England Medical Center, Boston, Massachusetts*
[4]*Division of General Medicine, Department of Medicine, Tufts University and New England Medical Center, Boston, Massachusetts*

Background and Objectives: This cross-sectional study of postoperative non-small cell lung cancer (NSCLC) patients examined possible effects of vitamin intake and folate status on disease-free survival.

Methods: Supplemental vitamin usage, dietary vitamin intake (Willett Food Frequency Questionnaire), red blood cell (RBC) folate, and serum folate concentrations were assessed in patients with a history of NSCLC. Exclusion criteria included factors that alter folate status or that are associated with altered nutritional habits: (1) evidence of cancer on history, physical, or chest radiograph; (2) tobacco, alcohol ingestion (>2 drinks/day), or cancer treatment within 3 months; (3) use of folate antagonists; and (4) age <60 years.

Results: 36 subjects were evaluated. The median disease-free censored survival was 24 months (range 4–41). Nineteen of 36 patients (53%) reported vitamin supplementation. Vitamin users had a longer median censored survival compared with nonusers (41 months versus 11 months; $P = 0.002$). With adjustment for cancer stage, the association between RBC folate and censored survival ($r = 0.35$; $P = 0.055$) and between serum folate and censored survival ($r = 0.32$; $P = 0.083$) approached statistical significance.

Conclusions: NSCLC patients who took vitamin supplements were more likely to be long-term survivors in the patients studied; a similar trend toward long-term survival was seen among patients with higher circulating folate concentrations. *J. Surg. Oncol. 1998;68:231–236.*

KEY WORDS: **non-small cell lung cancer; nutrition; folate; cancer prevention; vitamin supplementation**

INTRODUCTION

Epidemiologic evidence points to an inverse relationship between the intake of vegetables and fruits and the development of lung cancer in relatively healthy populations [1–5]. A recent randomized placebo-controlled trial underscored a similar relationship among postoperative non-small cell lung cancer (NSCLC) patients [6]. In this study, Pastorino et al. [6] examined the use of 300,000 IU of vitamin A in patients who had undergone

*Correspondence to: Aminah Jatoi, MD, Division of Clinical Nutrition, Department of Medicine, Tufts University and New England Medical Center, 711 Washington Street, Boston, MA 02111.
E-mail: jatoi_bf@hnrc.tufts.edu
Accepted 12 May 1998

surgical resection of stage I NSCLC. Patients who received high-dose vitamin A developed fewer second lung cancers; the time to development of second cancers (most of which were lung cancers) was significantly shorter in the placebo-treated group ($P = 0.045$). Patients treated with vitamin A may also have had a slight survival advantage when compared with placebo-treated patients ($P = 0.054$). Not all vitamin or micronutrient intake is beneficial, however. Two other intervention studies found a significant increase in lung cancer incidence in male smokers receiving β-carotene supplementation at doses of either 20 or 30 mg/day [7,8]. Taken together, these studies underscore the fact that nutrient intake plays an important, although not well-defined, role in the prevention and development of lung cancer.

For patients who have undergone surgical resection of early-stage NSCLC, dietary intake—specifically vitamin intake—is therefore an issue of interest. The 5-year mortality rate associated with this malignancy, even after surgical resection, exceeds 50%, with most patients succumbing to either metastatic disease or a second lung cancer [9]. Yet despite this high mortality, some patients do well. We hypothesized that habitual vitamin intake after cancer resection may in part explain this favorable outcome. We also hypothesized that long-term survivors of early-stage NSCLC have different dietary habits—which may protect them from recurrent cancer—as compared with most early-stage NSCLC patients who die from their disease. We therefore chose to examine the vitamin intake of a cross section of our NSCLC patient population in an effort to determine possible effects of nutritional habits on patients' disease-free survival.

MATERIALS AND METHODS

Overview

Supplemental vitamin usage, overall dietary vitamin intake, red blood cell (RBC) folate, and serum folate concentrations were assessed in patients who had undergone resection of early-stage NSCLC. Overall dietary vitamin intake was assessed with the Willett Food Frequency Questionnaire [10], for which correlations between energy-adjusted intake and plasma nutrient concentrations are strong and statistically significant for vitamins with reported cancer prevention properties, including folate, the carotenoids (includes multiple carotenoids in addition to β-carotene), vitamin E, and vitamin C ($r = 0.63$, $P < 0.01$; $r = 0.37$, $P < 0.01$; $r = 0.53$, $P < 0.01$; and $r = 0.44$, $P < 0.01$, respectively [11]). In order to provide supplemental information on self-reported dietary intake results, folate status was assessed biochemically. RBC and serum folate were chosen as markers for this purpose because (1) blood folate levels are stable biochemically while in storage at −70°C [12]; (2) in two separate studies, Heimburger et al. [13] and Saito et al. [14] suggested folate may directly prevent lung cancer by reversing bronchial squamous metaplasia; (3) greater intake of folate-rich foods has been shown to be associated with longevity in the elderly [15]; and (4) folate is abundant in vegetables and fruits [16].

Recruitment

Between September 1996 and June 1997, postoperative NSCLC patients in the Lung Tumor Evaluation Center at the New England Medical Center were randomly approached for participation in a research study requiring blood drawing and completion of a food frequency questionnaire. Fifty-one patients agreed to participate in the study protocol, which had been approved by the Human Investigation Review Committee at New England Medical Center, Boston, Massachusetts.

The present report is a nested analysis within this much larger, ongoing research effort. From the initial pool of 51 patients, 36 were included in the present analysis. The remainder were excluded because they possessed one or more of the following exclusion criteria: (1) evidence of cancer on history, physical examination, or chest radiograph; (2) tobacco use within the preceding 3 months; (3) use of folate antagonists, such as methotrexate, phenytoin, oral contraceptives, or sulfonamide antibiotics; (3) alcohol use of >2 drinks/day; or (4) cancer treatment, including surgery, within the preceding 3 months. The foregoing exclusion criteria were chosen because each has been shown to significantly alter folate status [17–20]. Subjects less than 60 years of age were also excluded to minimize the known confounding effects of age on dietary habits and micronutrient supplementation [21].

Vitamin Ingestion

At initial recruitment, subjects were questioned about their use of vitamin supplements. Subjects were asked whether they were currently taking supplemental vitamins, what type of vitamins they were taking, and whether these supplements were being taken to correct a known vitamin deficiency.

Dietary Intake

Subjects completed the Willett Food Frequency Questionnaire at the time of initial recruitment. They were also given the Food Models from the National Dairy Council (Courtesy of the National Dairy Council, Rosemont, IL), a pictorial description of portion sizes, to assist them in the completion of the food frequency questionnaire. Subjects had the option of completing the questionnaire at home. If questionnaires were not re-

ceived within 2 weeks after initial recruitment, subjects were contacted once by telephone as a reminder.

Assay Methods

Blood samples for RBC and serum folate were collected in vacuum tubes both with EDTA and without additives. Samples were immediately refrigerated after collection. Blood samples were later processed by mixing 100 μl of whole blood from each EDTA tube with 1 ml of 0.4% ascorbic acid and then stored at −70°C. Serum samples were also stored at −70°C. Assays for RBC and serum folate were run together at a later date with radioimmunoassay (RIA) kits (Bio-Rad, Hercules, CA).

Data Analysis

Results are expressed as means with standard deviations (SD) or as median values with ranges. We defined disease-free censored survival as the time in months from initial staging and treatment of the tumor to the time of study recruitment and participation, at which time all patients who were eligible for the study were found to be disease free. All survival data are censored because all subjects were alive and disease free at analysis. Data were log-transformed when not normally distributed. The Mann Whitney U-test was used to compare continuous data between two groups. Analysis of covariance was used to examine relationships between continuous data when adjustments for categorical or other continuous variables were necessary. All *P*-values are 2-tailed. *P*-values of < 0.05 were considered statistically significant. Analyses were performed with Systat 5.2.1 for Macintosh (SPSS, Chicago, IL).

RESULTS

Subject Characteristics

Of the 36 patients evaluated, the mean age was 71 ± 5 (mean ± SD). The male: female ratio was 17:19. The median postoperative disease-free censored survival was 24 months (range: 4 to 141 months). Subjects' cancer stage, as defined by Mountain's pathologic staging criteria [22], is distributed as follows: IA (16; 44%); IB (10 (28%); IIB (5; 14%); IIIA (2; <1%); and IIIB (2; <1%). One subject's cancer was unable to be accurately staged pathologically. Twenty-nine subjects (81%) completed and returned the food frequency questionnaire.

Vitamin Usage

Nineteen of the 36 patients (53%) reported regular supplemental vitamin usage. The vitamin supplements taken are listed in Table I. No patient reported taking vitamins to treat a known vitamin deficiency. The male to female ratio among the vitamin users was 8:11, whereas among the nonusers, it was 9:8. The mean age ± SD of the vitamin users was 72 ± 6 years, whereas among the vitamin nonusers, it was 71 ± 5 years. Vitamin users had the following tumor stages: IA (4), IB (7), IIB (5), IIIA (2), and IIIB (1). Tumor stages among vitamin nonusers are as follows: IA (12), IB (3), and IIIB (1) with one patient unable to be pathologically staged. Vitamin users had significantly longer median censored disease-free survivals compared with nonusers: 41 months (range 4–141) versus 11 months (range 4–84) ($P = 0.002$) (Fig. 1). A significant survival advantage continued to be seen after adjustment for cancer stage ($P = 0.04$).

TABLE I. Reported Vitamin Supplementation Among Non-Small Cell Lung Cancer Patients

Vitamin	% of subjects who reported use
Multivitamin	39
Folate	3
Vitamin E	14[a]
Vitamin C	14[b]

[a]Two of these subjects reported taking a multivitamin and four also reported vitamin C supplementation.
[b]Two of these subjects reported taking a multivitamin, and four also reported vitamin E supplementation.

Dietary Intake

Dietary intake was analyzed for the 29 patients who returned the food frequency questionnaire. Vitamin intake was examined as total vitamin intake, dietary vitamin intake with the exclusion of vitamin supplementation, and vitamin intake adjusted for energy consumption. With adjustment for cancer stage, no significant relationships were seen between reported intake of folate, the carotenoids, vitamin E, and vitamin C and censored survival. In addition, among the 29 patients who returned the dietary surveys total caloric intake was no different between the patients who reported being vitamin users and those who reported being nonusers.

Folate Status

The mean RBC and serum folate concentrations were 358.5 ± 201.6 and 12.8 ± 9.2, respectively, within the group. The normal ranges for these parameters with the methodology used in the present study are 215–1,291 ng/ml for RBC folate and 1.5–20.6 ng/ml for serum folate. These normative values are supplied by the manufacturer of the RIA kit and are based on a calculated mean concentration ± 2 SD for both RBC and serum folate from 299 samples taken from healthy blood donors.

With adjustment for cancer stage, the association between RBC folate and censored survival approached statistical significance ($r = 0.35$; $P = 0.055$) (Fig. 2). With the same adjustment, the association between serum folate and censored survival also approached statistical significance ($r = 0.32$; $P = 0.083$) (Fig. 3).

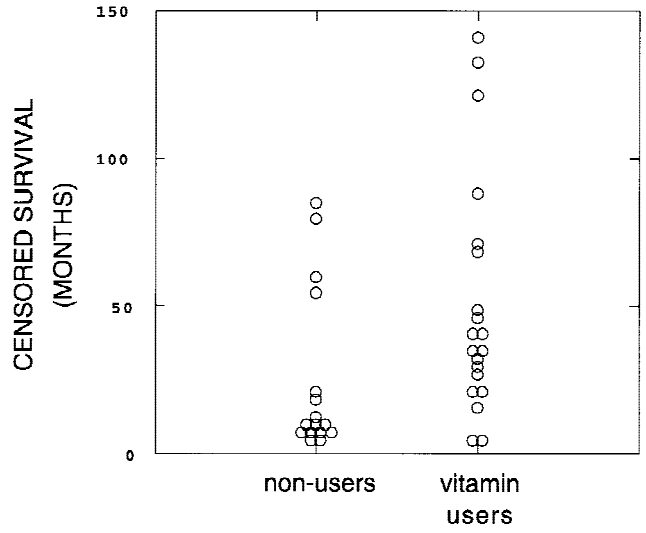

Fig. 1. Subjects who reported using vitamin supplements had a significantly longer median censored survival compared with nonusers: 41 months (range 4–141 months) versus 11 months (range 4–84 months) ($P = 0.002$).

CONCLUSIONS

In this cross-sectional study, subjects who took vitamin supplements and were more folate-replete by biochemical assessment were more likely to be long-term survivors within the group. These findings suggest that the nutritional habits of these cancer survivors may be protecting them from recurrent cancer, although the design of this study precludes any definitive statement on causality. Although the reported dietary intake of micronutrients within our study did not demonstrate correlations with respect to censored survival, this result can be explained by the inherent imprecision seen with reported dietary intake data coupled with the fact that our sample size was relatively small. Within the group of patients we studied, however, our results clearly demonstrate that the use of vitamin supplements was associated with long-term survivorship.

The RBC and serum folate results are provocative, despite the fact that they fall short of conventional levels of statistical significance. Although early-stage NSCLC carries a high mortality, several of the short-term survivors within our group of patients will nonetheless become long-term survivors. The data from the short-term censored survivors in this study are therefore ''contaminated'' by those patients who will become long-term survivors. Because this group is skewing our RBC and serum folate results, we believe the clinical significance of our findings may exceed their statistical significance. Future longitudinal studies with larger numbers of subjects should determine whether folate-replete patients are more likely to become long-term survivors after resection of early-stage NSCLC than are those with diminished folate status.

Finally, our study has several limitations. First, as alluded to, our data are cross-sectional and require a prospective study for confirmation. Even with regard to vitamin supplementation, the cross-sectional design of our study is problematic in that it does not allow for accurate assessment of duration of use. Second, although we randomly approached patients for enrollment, we may nonetheless have evaluated a biased population. Vitamin ingestion and a willingness to seek regular medical checkups may be two variables that co-migrate within a

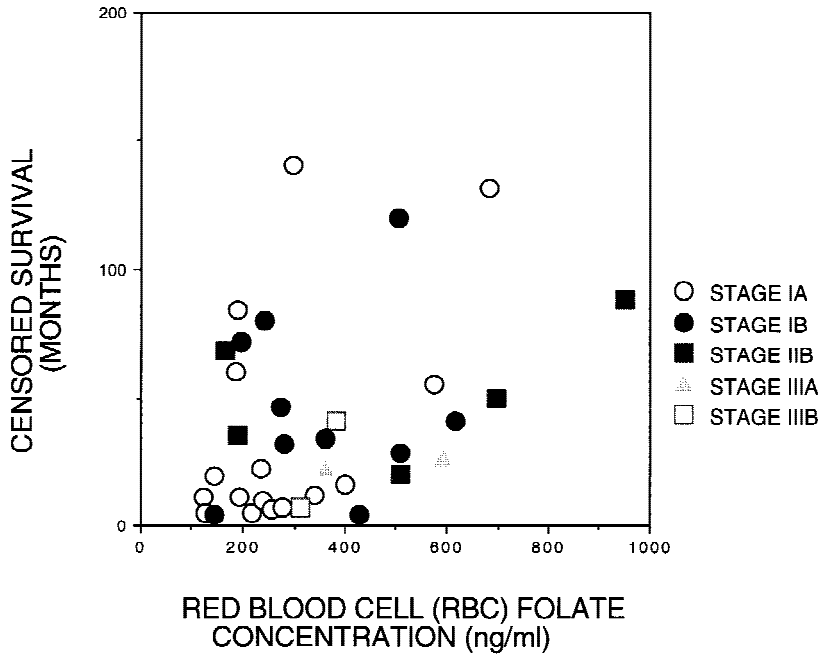

Fig. 2. After adjustment for cancer stage, the association between red blood cell (RBC) folate and censored survival approached statistical significance ($r = 0.35$; $P = 0.055$). One patient whose cancer could not be accurately pathologically staged was included in the analysis, but not in the graph.

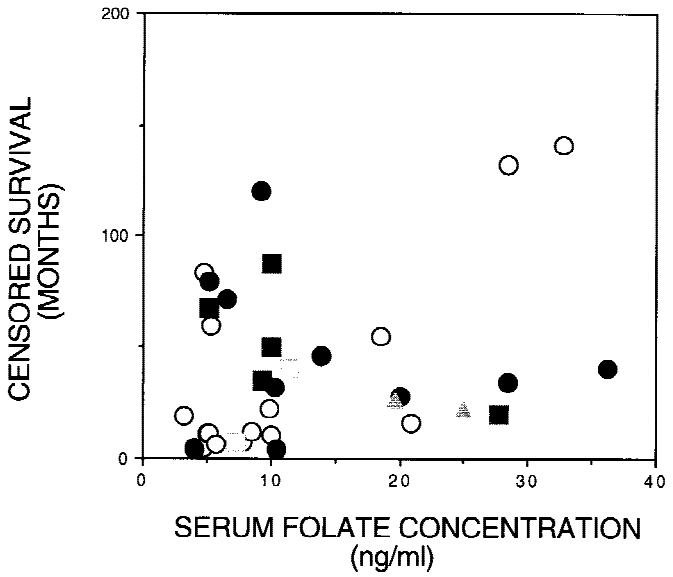

Fig. 3. After adjustment for cancer stage, the association between serum folate and censored survival approached statistical significance ($r = 0.32$; $P = 0.083$). One patient whose cancer could not be accurately pathologically staged was included in the analysis, but not in the graph.

population. Large cross-sectional databases have observed that a variety of other cancer preventive habits do, in fact, co-migrate with supplemental vitamin usage [23]. As mentioned earlier, a prospective study with aggressive recruitment and follow-up of all patients who undergo resection of early-stage NSCLC is necessary to overcome this potential for bias. Lastly, our study did not evaluate pharmacologic doses of vitamin A, which has been demonstrated to reduce the risk of second lung cancers postoperatively [6]. We chose not to focus on vitamin A because the Willett Food Frequency Questionnaire does not provide accurate dietary information for this vitamin [11]. Perhaps one of the strengths of our study, however, is that by evaluating the vitamins we did and by asking open-ended questions with regard to supplementation, we found that the ingestion of a variety of vitamin supplements identify patients who are more likely to be long-term survivors. Thus, our study raises the possibility that multiple different nutrients may together promote long-term disease-free survival in early-stage NSCLC patients.

In summary, our study indicates that vitamin supplementation and perhaps folate intake are associated with long-term survival in postoperative, early-stage NSCLC patients. In view of the poor prognosis associated with this malignancy, further studies need to be performed to determine whether there is a causal relationship between vitamin use and long-term survival.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the laboratory assistance of members of the Nutritional Evaluation Laboratory at the Jean Mayer Human Nutrition Research Center on Aging at Tufts University. The authors also express their thanks to Helen Rasmussen, RD, for her help in collecting the Food Frequency Questionnaires.

REFERENCES

1. Menkes MS, Comstock GW, Vuilleumier JP, et al.: Serum beta-carotene, vitamins A and E, selenium, and the risk of lung cancer. N Engl J Med 1986;315:1250–1254.
2. Ziegler RG, Mason TJ, Stemhagen A, et al.: Carotenoid intake, vegetables, and the risk of lung cancer among white men in New Jersey. Am J Epidemiol 1986;123:1080–1093.
3. Nomura AMY, Stemmerman GN, Heilbrun LK, et al.: Serum vitamin levels and the risk of cancer of specific sites in men of Japanese ancestry in Hawaii. Cancer Res 1985;45:2369–2372.
4. Knekt P, Jarvinen R, Seppanen R, et al.: Dietary antioxidants and the risk of lung cancer. Am J Epidemiol 1991;134:471–479.
5. Ocke MC, Bueno-de-Mesquita HB, Feskens EJ, et al.: Repeated measurements of vegetables, fruits, beta carotene, and vitamins C and E in relation to lung cancer. Am J Epidemiol 1997;145:358–365.
6. Pastorino U, Infante M, Maioli M, et al.: Adjuvant treatment of stage I lung cancer with high-dose vitamin A. J Clin Oncol 1993; 11:1216–1222.
7. Anonymous: The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–1035.
8. Omenn GS, Goodman GE, Thornquist MD, et al.: Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular risk. N Engl J Med 1996;334:1150–1155.
9. Ginsberg RJ, Vokes EE, Raben A: Non-small cell lung cancer. In DeVita VT, Hellman S, Rosenberg SA (eds): ''Principles and Practice of Oncology.'' Philadelphia: Lippincott−Raven, 1997: 858–911.
10. Willett WC, Sampson L, Stampfer MJ, et al.: Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
11. Jacques PF, Sulsky SI, Sadowski JA, et al.: Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. Am J Clin Nutr 1993; 57:182–189.
12. Gunter EW, Twite DB: Improved materials for long-term quality control assessment of erythrocyte folate analysis. Clin Chem 1990;36:2139.
13. Heimburger DC, Alexander CB, Birch R, et al.: Improvement in bronchial squamous metaplasia in smokers treated with folate and B12. JAMA 1988;259:1525–1530.
14. Saito M, Kato H, Tsuchida T, Konaka G: Chemoprevention effects on bronchial squamous metaplasia by folate and B12 in heavy smokers. Chest 1994;106:496–499.

15. Magni E, Bianchetti A, Rozzini R, Trabucchi M: Influence of nutritional intake on 6-year mortality in an Italian elderly population. J Nutr Elderly 1994;13:25–34.
16. Groff JL, Gropper SS, Hunt SM: The water-soluble vitamins. In ''Advanced Nutrition and Human Metabolism.'' Minneapolis–St Paul: West Publishing, 1995:262–269.
17. Schreurs WHP, Odink J, Egger RJ, et al.: The influence of radiotherapy and chemotherapy on the vitamin status of cancer patients. Int J of Vitam Nutr Res 1985;55:425–432.
18. Clamon GH, Feld R, Evans WK, et al.: Serum folate and vitamin B_{12} levels in patients with small cell lung cancer. Cancer 1984; 53:306–310.
19. Pyathilake CJ, Macaluso M, Hine RJ, et al.: Local and systemic effects of cigarette smoking on folate and vitamin B12. Am J Clin Nutr 1994;60:559–566.
20. Gloria C, Cravo M, Camilo ME, et al.: Nutritional deficiencies in chronic alcoholics in relation to dietary intake and alcohol consumption. Am J Gastroenterol 1997;92:485–489.
21. Subar AF, Block G: Use of vitamin and mineral supplements: Demographics and amounts of nutrients consumed. Am J Epidemiol 1990;132:1091–1101.
22. Mountain CF: Revisions in the international staging system for lung cancer. Chest 1997;111:1710–1717.
23. Patterson RE, Neuhouser ML, White E, et al.: Cancer-related behavior for vitamin supplement users. Cancer Epidemiol Biomarkers 1998;7:79–81.