Open Journal of Gastroenterology, 2011, 1, 28-34 **OJGas**
doi:10.4236/ojgas.2011.12005 Published Online November 2011 (http://www.SciRP.org/journal/ojgas/).

Effect of zinc supplementation in patients with type C liver cirrhosis

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Received 16 September 2011; revised 25 October 2011; accepted 4 November 2011.

ABSTRACT

Zinc is often deficient in patients with liver cirrhosis, and treatment with zinc provides short-term improvement in protein metabolism. However, the long-term effects of zinc have not been fully clarified. The present study aimed to analyze the effect of zinc on the long-term clinical course, especially hepatocarcinogenesis, in type C liver cirrhosis. Among patients with type C liver cirrhosis visiting our hospital between June 1998 and January 2009, those with a serum albumin level ≤ 3.5 g/dL and a serum zinc level ≤ 70 μg (1.07 μmol)/dL were selected. Thirty-seven patients were randomly divided into 2 groups: group B (12 g/day branched-chain amino acid granules) and group BZ (same as group B plus 100 mg/day - 600 mg/day zinc sulfate or 150 mg/day - 225 mg/day polaprezinc). Multivariate analysis revealed that the administration of zinc was not a significant determinant, but pretreatment serum zinc levels (hazard ratio [HR], 0.921; 95% confidence interval [CI], 0.853 - 0.994) and serum zinc levels less than 80 μg (1.22 μmol)/dL 12 months after beginning this study (HR, 6.866; 95% CI, 1.399 - 33.707) were significant determinants of carcinogenesis and death. Serum albumin levels in patients whose serum zinc levels had not increased up to 80 μg/dL by the third year of this study were significantly lower (p = 0.023) than those of patients that had increased up to 80 μg/dL. *Conclusions*: In type C liver cirrhosis with zinc deficiency, administration of zinc does not improve cancer-free survival. However, serum zinc levels can predict outcomes in patients with type C liver cirrhosis. However, although zinc may play a role in hepatocarcinogenesis, the precise implications remain to be clarified.

Keywords: Hepato-Carcinogenesis; Trace Element; Protein Metabolism

1. INTRODUCTION

Primary liver cancer is one of the leading causes of death from cancer [1,2]. Hepatitis B and C viruses play an important role in the pathology of hepatocellular carcinoma [2-4]. Although antiviral treatment regimens such as combined Peg-interferon plus ribavirin therapy for hepatitis C and nucleic acid analogue therapy for hepatictis B have lowered the incidence of liver cancer, these regimens do not completely prevent carcinogenesis [2,5, 6]. Factors other than hepatitis viruses also play important roles in the pathogenesis of hepatocellular carcinoma. Conditions involving metabolic abnormalities such as nonalcoholic fatty liver disease, obesity, and diabetes mellitus have been suggested to be risk factors for liver carcinogenesis [2,7-10]. Liver cirrhosis patients have been shown to have abnormal amino acid balance due to branched chain amino acid (BCAA) deficiency, and replacement of BCAAs has been shown to improve event-free survival and liver function in patients with liver cirrhosis [9,10]. These findings suggest that abnormal amino acid metabolism may aggravate liver cirrhosis. In addition, administration of BCAAs has been shown to significantly suppress liver carcinogenesis in obese individuals with a body mass index of at least 25 [9,10]. Therefore, metabolic abnormalities associated with liver disease are involved in various pathological conditions; however, their mechanisms have not been fully clarified.

Zinc, which is a trace metal, is indispensable for growth and differentiation of cells, and is one of the most important nutrients of metabolism in humans [11]. More than 300 proteins possess a zinc-containing region,

and these proteins play an important role in the regulation of cell function [12-15]. Therefore, Zinc may be closely involved in many bodily functions. Homeostasis of zinc in vivo is primarily maintained by a balance between zinc-binding metallothionein protein and the expression of 2 key zinc transporters [16-20]. Deficiency of zinc can lead to growth disorders, cognitive disorders, and compromised immune function [21-24]. Furthermore, zinc deficiency can accompany liver cirrhosis, leading to abnormal levels of ammonia and other substances due to abnormal protein metabolism, and the onset of hepatic encephalopathy [25,26]. Zinc replacement has been shown to alleviate hepatic encephalopathy and hyperammonemia and to improve protein metabolism [25,26]. We previously showed that combined BCAA plus zinc treatment is more effective in alleviating abnormal ammonia metabolism and abnormal amino acid balance than BCAA monotherapy [27]. However, there are very few reports on the long-term efficacy of zinc replacement therapy [28,29]. In this study, we analyzed the effect of zinc supplementation and the relationship between serum zinc levels and the long-term course, especially hepatocarcinogenesis, of hepatitis C virus-related liver cirrhosis.

2. MATERIALS AND METHODS

2.1. Subjects

Among the patients with hepatitis C virus-related liver cirrhosis visiting our facility between June 1998 and January 2009, those satisfying all the following criteria were enrolled in the study: (1) serum albumin levels no higher than 3.5 g/dL; (2) serum zinc levels no higher than 70 μg (1.07 μmol)/dL; (3) patients were able to receive periodical outpatient care, and (4) patients provided informed consent to the study. Patients who were already treated with BCAA granules, those with a positive history of liver cancer, those having esophageal or gastric varix, which needed to be treated, and patients scheduled to receive antiviral therapy (e.g., interferon) during the study period were excluded from the study. Other exclusion criteria included coinfection with hepatitis B virus or human immunodeficiency virus. Patients who developed liver cancer within 6 months after the start of the study were also excluded.

2.2. Protcol

These 37 subjects were randomized into 2 groups: group B (n = 16, treatment with 12 g/day BCAA granules; Livact, Ajinomoto Pharmaceutical Co., Ltd., Tokyo, Japan) and group BZ (n = 21, treatment with 12 g/day BCAA granules plus zinc preparation). In group BZ, the zinc preparation initially used was 600 mg/day zinc sulfate (containing 136 mg zinc, 2.08 mmol zinc) for patients with a serum zinc level no higher than 50 μg (0.76 μmol)/dL and either 200 mg/day zinc sulfate (containing 45 mg zinc, 0.69 mmol zinc) or 150 mg/day polaprezinc (containing 34 mg zinc, 0.52 mmol zinc; Zeria Pharmaceutical Co., Ltd., Tokyo, Japan) for patients with serum zinc levels between 50 and 70 μg (0.76 μmol and 1.07 μmol)/dL. If serum zinc levels did not reach 80 μg (1.22 μmol)/dL, the zinc sulfate dose was later increased up to 600 mg (2.08 mmol)/day. If serum zinc levels exceeded 120 μg (1.83 μmol)/dL, the zinc sulfate dose levels were reduced. Zinc dose levels were also reduced in patients with complaints of nausea or anorexia after the start of zinc treatment. The background variables of the patients are summarized in **Table 1**. There were no serious adverse events during the study. There were no significant differences in any background variables between the 2 groups (**Table 1**). In this open-label clinical study, the subjects were randomly allocated to groups B and BZ at a ratio of 1:1 in the following manner. All patients visiting our hospital were assigned serial case numbers for the purpose of identification. Among the patients who had provided informed consent to the study, those who had even case numbers were allocated to group BZ and those who had odd case numbers were allocated to group B. The patients were followed up at intervals of 2 - 3 months by means of a hematological test and abdominal ultrasonography or computed tomography/magnetic resonance imaging. The primary endpoint of this study was cancer free survival. The follow-up was completed upon diagnosis of liver cancer or death. The follow-up period (mean ± SD) was 1152 ± 698 days. During the study period, prophylactic endoscopic treatment of varix

Table 1. Patient data.

	Group B (n = 16)	Group BZ (n = 21)	P
Age (years)	64.9 ± 10.3	68.7 ± 6.5	0.4807
Sex (male/female)	8/8	11/10	0.8859
Body Mass Index	23.5 ± 3.0	24.0 ± 3.5	0.7359
T.Bil (mg/dL)	0.98 ± 0.35	1.20 ± 0.55	0.2503
Albumin (g/dL)	3.4 ± 0.2	3.3 ± 0.2	0.3498
Zinc (μg/dL)	59.5 ± 9.8	58.0 ± 8.6	0.4619
Fischer ratio	1.92 ± 0.54	1.58 ± 0.48	0.0806
Ammonia (μg/dL)	36.4 ± 14.4	49.6 ± 29.4	0.2088
PT (%)	78.6 ± 14.2	72.1 ± 13.9	0.1042
ALT (IU/L)	61.6 ± 37.5	61.5 ± 29.0	0.8301
WBC (/μL)	4019 ± 1212	3881 ± 922	0.8181
Hb (g/dL)	12.5 ± 1.7	12.6 ± 1.4	0.7709
Platelets ($\times 10^4$/μL)	12.0 ± 6.8	9.5 ± 3.2	0.5500
FBS (mg/dL)	107.4 ± 17.1	97.1 ± 10.4	0.0554
AFP (ng/ml)	35.8 ± 40.9	48.0 ± 82.4	0.9755

Values are expressed as mean ± s.d. PT, prothrombin time. ALT, alanine transaminase. FBS, fasting blood sugar. AFP, alfa-feto protein. P value between two groups was calculated by Man-Whitney *U* test, except sex. P value of sex was calculated by chi-square test.

and treatment of ascites or edema with diuretics, were administered as needed. In addition, rupture of esophageal/gastric varix did not occur in any patient, because risky varices were prophylactically treated. Inter-group comparison was performed to determine the time dependent changes in parameters such as age, sex, body mass index, total bilirubin, serum albumin levels, prothrombin time (PT), aspartate transaminase (AST) levels, alanine transaminase (ALT) levels, ammonia levels, platelet count, Fischer ratio (FR), pretreatment serum zinc levels, and fasting serum glucose levels during the first 3 years of treatment. We also compared use/non-use of zinc preparation (BZ vs. B) and response/no response to serum zinc levels (serum zinc levels of 80 μg (1.22 μmol)/dL or higher 12 months after the start of treatment/serum zinc levels less than 80 μg/dL 12 months after the start of treatment). This clinical study was approved by the institutional review board, and all patients provided consent to the study either orally or in writing.

2.3. Statiscal Analysis

Statistical analysis was performed using the computer program Dr. SPSS II for Windows (SPSS Co., Ltd., Tokyo, Japan). We used the Mann-Whitney *U* test or chi-square test for inter-group comparisons of background variables. The cumulative cancer-free survival rate of each group was determined by the Kaplan-Meier method. The significance of differences was analyzed by log rank test. The Cox proportional hazards model was used for evaluating the hazard ratio with regard to carcinogenesis and death.

3. RESULTS

3.1. Comparison of Serum Zinc Levels between the Groups

Serum zinc levels were significantly increased at 1 years - 3 years after treatment in group BZ compared with those in group B (**Figure 1**).

3.2. Factors Associated with Cancer-Free Survival

First, we performed univariate analysis of factors asso ciated with carcinogenesis and death by using a Cox hazards model. The results of this analysis are shown in **Table 2**. Among the factors analyzed, 5 factors (response to serum zinc levels, FR, PT, platelet count, and pre-treatment serum zinc levels) had a p value ≤ 0.10. Multivariate analysis using these 5 factors indicated that the response to serum zinc levels and pre-treatment serum zinc levels were significant factors, with the former being the most potent factor (**Table 3**). Therefore, we compared the cumulative cancer-free survival rate between group H (patients with elevated serum zinc levels more than 80 μg/dl after the administration of zinc preparations) and group L (other patients including patients who did not receive zinc supplementation) (**Figure 2**). The incidence of cancer and the death rate were significantly lower in group H than those in group L (p = 0.0432, log rank test). We compared the background variables between groups H and L (**Table 4**). Fasting blood sugar (FBS) of group H was significantly lower than in group L.

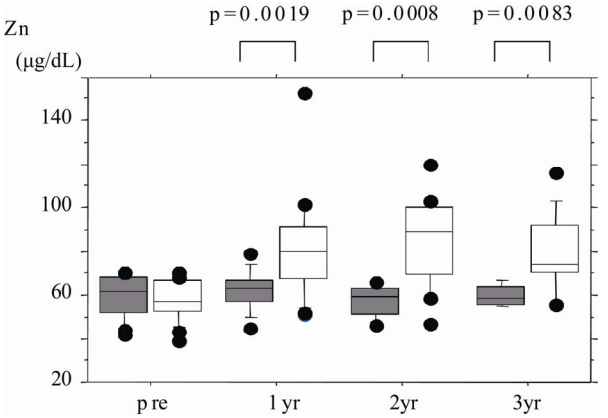

Figure 1. Time course of serum zinc levels during 3 years of treatment (box plot; median, 10th - 90th percentile and 25th - 75th percentile; circles show outliers). Comparison between group B (gray boxes) and group BZ (white boxes). Zinc levels were significantly higher in Group BZ than those in Group B throughout the 3-year period.

Table 2. Factors associated with hepatocarcinogenesis and death by univariate analysis.

Factor	HR	95%CI	p
Age (years)	0.984	0.929 - 1.042	0.587
Sex (male/female)	0.797	0.258 - 2.458	0.692
Body Mass Index	1.066	0.905 - 1.255	0.446
T.Bil (mg/dL)	2.200	0.643 - 7.521	0.209
Albumin (g/dL)	0.273	0.025 - 3.010	0.289
PT (%)	0.936	0.882 - 0.933	0.028
AST (IU/L)	0.985	0.946 - 1.006	0.150
ALT (IU/L)	0.990	0.972 - 1.008	0.266
FBS (mg/dL)	0.987	0.951 - 1.085	0.500
Platelets ($\times 10^4/\mu L$)	0.796	0.649 - 0.976	0.029
Fischer ratio	0.261	0.1 - 1.124	0.071
Pre-treatment Zinc (μg/dL)	0.932	0.871 - 0.996	0.037
Zinc at 12-month < 80 (μg/dL)	4.161	0.928 - 18.650	0.062
BZ (vs B)	0.957	0.320 - 2.864	0.937

HR, hazard ratio. CI, confidence interval. PT, prothrombin time. FR, Fischer ratio. AST, aspartate transaminase. ALT, alanine transaminase.

Table 3. Factors associated with hepatocarcinogenesis and death by multivariate analysis.

Factor	HR	95%CI	p
Zinc at 12-month < 80 (μg/dL)	6.866	1.99 - 33.707	0.010
Pre-treatment Zinc (μg/dL)	0.921	0.853 - 0.994	0.043
PT (%)	0.946	0.94 - 1.002	0.058

3.3. Time Course of Changes in Hematological Parameters

Serum albumin levels tended to be higher in group H than in group L during the second year and were significantly higher during the third year (p = 0.023, **Figure 3(a)**). No significant differences were observed in PT and ALT levels between the 2 groups (data not shown). We estimated the percentage change in serum ammonia levels from the pretreatment baseline levels and found that serum ammonia levels tended to decrease after the start of treatment in group H and were significantly lower in the third year compared with those in group L (**Figure 3(b)**). Platelet count was not significantly different between the 2 groups (data not shown). The FR was significantly higher in group H than that in group L during the third year (p = 0.035, **Figure 3(c)**).

4. DISCUSSION

Factors known to be involved in carcinogenesis from chronic liver disease include hepatitis viruses, alcohol consumption, and smoking [2,30]. In addition, several metabolic factors have recently been shown to be associated with liver carcinogenesis. Obesity and diabetes mellitus are risk factors for liver carcinogenesis [2], and it has been suggested that iron is involved in carcinogenesis in patients with hepatitis C virus-related chronic liver disease [31]. In patients with chronic viral liver disease, antiviral therapy (e.g., interferon therapy for chronic hepatitis C and nucleic acid analog therapy for hepatitis B virus-related chronic liver disease) has been shown to suppress carcinogenesis; however, antiviral therapy alone cannot completely prevent liver carcino genesis in these patients [2,5,6]. Furthermore, as was the case in this study, in hepatitis C virus-related chronic

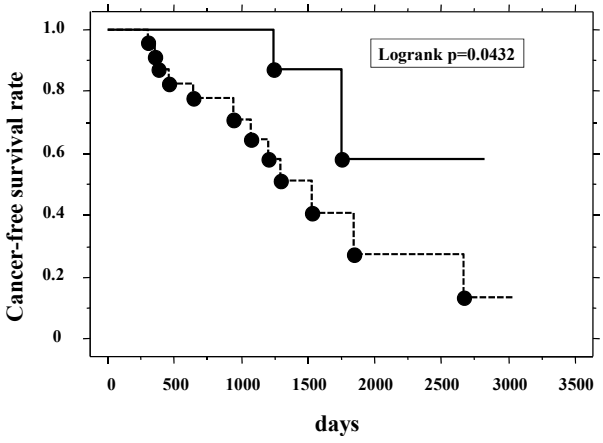

Figure 2. Comparison of cumulative cancer-free survival rate between group H (solid line) and group L (dotted line) (Kaplan-Meier method). The cancer-free survival rate was significantly higher in group H than that in group L (log rank test).

Table 4. Patient data.

	Group H (n = 13)	Group L (n = 24)	p
Age (years)	57.5 ± 9.9	68.8 ± 8.9	0.8362
Sex (male/female)	7/6	12/12	0.8231
Body Mass Index	23.2 ± 3.7	24.1 ± 3.0	0.5241
T.Bil (mg/dL)	1.09 ± 0.65	1.12 ± 0.38	0.4940
Albumin (g/dL)	3.4 ± 0.2	3.4 ± 0.2	0.4172
Zinc (g/dL)	57.3 ± 8.9	59.6 ± 9.2	0.3646
Fischer ratio	1.68 ± 0.54	1.76 ± 0.53	0.8114
Ammonia (μg/dL)	54.8 ± 35.5	36.0 ± 14.0	0.1522
PT (%)	76.2 ± 16.1	74.3 ± 13.4	0.5886
ALT (IU/L)	58.8 ± 24.2	64.0 ± 36.6	0.9746
WBC (/μL)	4008 ± 911	3904 ± 1126	0.7026
Hb (g/dL)	12.7 ± 1.3	12.5 ± 1.6	0.6332
Platelets ($\times 10^4$/μL)	10.7 ± 3.3	10.5 ± 6.0	0.2391
FBS (mg/dL)	94.8 ± 10.4	105.2 ± 15.2	0.0386
AFP (ng/ml)	26.2 ± 29.2	51.7 ± 79.8	0.3992

Values are expressed as mean ± s.d. PT, prothrombin time. ALT, alanine transaminase. FBS, fasting blood sugar. AFP, alfa-feto protein. P value between two groups was calculated by Man-Whitney *U* test, except sex. P value of sex was calculated by chi-square test.

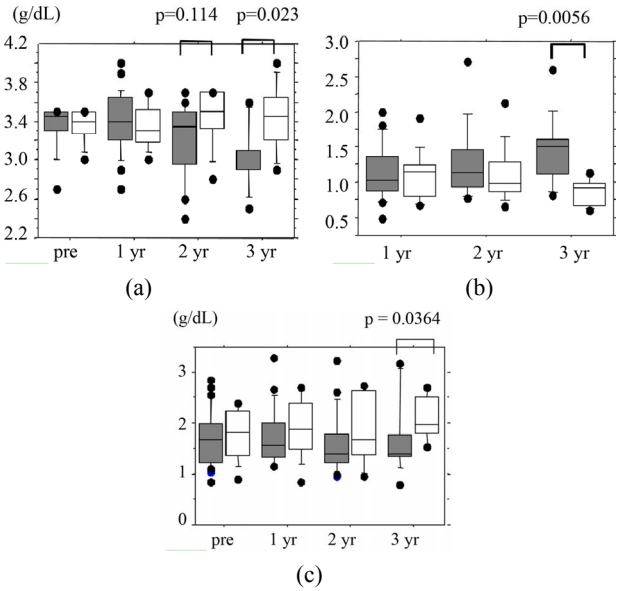

Figure 3. Time course of serum levels during 3 years of treatment (box plot; median, 10^{th} - 90^{th} percentile and 25^{th} - 75^{th} percentile, circles show outliers). (a): Comparison of serum albumin levels between group B (gray boxes) and group H (white boxes). Albumin levels were significantly higher in group H than those in group L in the third year. Statistical analysis was performed by Mann-Whitney *U* test; (b): The percent change in serum ammonia levels from pretreatment levels in group L (gray boxes) and group H (white boxes). Group H showed a significantly greater percent reduction than group L. Statistical analysis was performed by Mann-Whitney *U* test; (c): Comparison of FR between group L (gray boxes) and group H (white boxes). The FR was significantly higher in group H than that in group L in the third year. Statistical analysis was performed by Mann-Whitney *U* test. FR, Fischer ratio.

liver disease with advanced liver fibrosis, interferon therapy is often difficult because of complications such as thrombopenia [32,33]. Therefore, in addition to antiviral therapy, it is important to investigate metabolic factors that possibly suppress carcinogenesis.

It has been shown that zinc, which is a trace metal, is deficient in patients with chronic liver disease and is involved in metabolic abnormalities primarily pertaining to ammonia; these metabolic abnormalities can be alleviated by zinc replacement [25,26]. However, to date, the relationship between long-term effects of patients with liver disease and the metabolic function of zinc has not been sufficiently studied.

In the present study, administration of zinc preparations did not significantly affect cancer-free survival. However, in patients with elevated serum zinc levels (≥80 μg/dL, ≥1.22 μmol/dL) after the administration of zinc preparations, the rate of hepatocarcinogenesis and death was significantly reduced, suggesting that the serum zinc level might be an important predictor of cancer-free survival. Although the present study was preliminary, it showed for the first time that serum zinc levels are a crucial predictor of clinical outcomes. Matsuoka *et al*. also studied the effect of long-term zinc supplementation, and showed that there were improved clinical outcomes in patients whose serum zinc levels were increased [29]. However, the dose of zinc administered to patients in their study was small compared with that in the present study (34 mg vs. 136 mg zinc, 0.52 mmol vs. 2.08 mmol), and they did not show a relationship between the serum level of zinc and clinical outcomes.

The exact mechanism of zinc for the improvement of cancer-free survival is still debatable. One possible explanation is that the effect of zinc might be due to improved nitrogen metabolism such as serum albumin levels and FR in group H. Zinc is known to be involved in metabolism of ammonia as a coenzyme of ornithine transcarbamylase (one of the enzymes of the hepatic urea cycle) [26,34]. In patients with chronic liver disease, the ability of detoxicating ammonia is reduced because of zinc deficiency, and this can be improved by zinc replacement [25,26]. Because ammonia is also metabolized in skeletal muscles by consuming BCAAs, improvement in ammonia detoxification in the liver is expected to reduce BCAA consumption. This can lead to alleviation of BCAA deficiency and improvement in amino acid imbalance [27,34]. Indeed, we previously showed that zinc treatment contributes to improving amino acid imbalance [27]. Administration of BCAAs as a drug improves the prognosis of patients with chronic liver disease [9,10], and survival is better in patients with a high serum FR [34]. Therefore, it is possible that the effects of zinc are due to an improvement in nitrogen metabolism. The fact that patients with liver cirrhosis may suffer from malnutrition and other deficiencies means that other possible reasons for the reduction in cancer-free survival should be considered. We compared other nutritional factors between group H (patients with serum zinc levels > 80 μg/dl (1.22 μmol/dl at 12-month of this study) and group L (other patients) (**Table 4**). There were no significant differences between the groups in terms of body mass index or hemoglobin, but FBS was significantly lower in group H than in group L. However, FBS was not significantly associated with cancer-free survival in univariate analysis (**Table 2**). Thus, although this study could not rule out the possibility that factors other than zinc might affect patients outcomes, serum zinc levels nonetheless appear to play some an important role in predicting cancer-free survival.

The magnitude of elevation in serum zinc levels after administration of zinc preparations differs among individuals. A transporter is involved in zinc absorption in the small bowel mucosa, and the expression of this transporter has been suggested to be affected by zinc exhaustion [17-20]. It is difficult to observe this effect during clinical practice, and it is unknown how to adjust serum zinc levels based on this effect. In the current study, the administered zinc level 12 months after the start of treatment was not significantly different between group H and group L. The reason why there was no elevation in serum zinc levels in some cases but sufficient elevation in others remains unknown and should be investigated in the future. However, an analysis of individual cases revealed an increase in zinc dose level and subsequent elevation in serum zinc levels, indicating that increasing zinc dose levels is one of the means to achieve elevated serum zinc levels. Unfortunately, in some patients, it is difficult to ingest sufficient amounts of zinc because of adverse events such as nausea. Therefore, it is desirable to develop drug preparations enabling easy intake of large amounts of zinc.

A limitation of the present study is the small sample size. However, to date, there have been few reports on the long-term clinical efficacy of zinc other than short-term data. Therefore, we believe that the data in the present study are important, and hopefully will lead to further large-scale studies.

In conclusion, our results showed that administration of zinc does not improve hepatocarcinogenesis. However, a serum zinc level greater than 80 μg (1.22 μmol)/dL after zinc supplementation might be a good predictor of cancer-free survival.

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