Perioperative arginine-supplemented nutrition in malnourished patients with head and neck cancer improves long-term survival1–5

Nikki Buijs, Marian AE van Bokhorst-de van der Schueren, Jacqueline AE Langius, C Rene Leemans, Dirk J Kuik, Mechteld AR Vermeulen, and Paul AM van Leeuwen

ABSTRACT
Background: Plasma arginine concentrations are lower in patients with cancer, which indicates that arginine metabolism may be disturbed in these patients. Arginine supplementation has been associated with positive effects on antitumor mechanisms and has been shown to reduce tumor growth and to prolong survival. Furthermore, the prognosis of patients with head and neck cancer remains disappointing. Insufficient intake frequently leads to malnutrition, which contributes to high morbidity and mortality rates.

Objective: The aim of this study was to assess the long-term effects of perioperative arginine supplementation in severely malnourished patients with head and neck cancer.

Design: In this double-blind, randomized, controlled trial, we randomly assigned 32 severely malnourished patients with head and neck cancer to receive 1) standard perioperative enteral nutrition (n = 15) or 2) arginine-supplemented perioperative enteral nutrition (n = 17). The primary outcome was long-term (≥10 y) survival. Secondary outcomes included the long-term appearance of locoregional recurrence, distant metastases, and secondary primary tumors.

Results: No significant differences in baseline characteristics were observed between groups. The group receiving arginine-enriched nutrition had a significantly better overall survival (P = 0.019) and better disease-specific survival (P = 0.022). Furthermore, the arginine-supplemented group had a significantly better locoregional recurrence-free survival (P = 0.027). No significant difference in the occurrence of distant metastases or occurrence of a second primary tumor was observed between the groups.


INTRODUCTION

Despite the available treatments, the survival of head and neck cancer patients remains disappointing because of uncontrollable persistent or recurrent disease. The incidence of postoperative complications within this population is 20–50%, which leads to prolonged hospital stays and a poorer prognosis. Malnutrition is one of the factors that contributes to disappointing morbidity rates. Most studies report malnutrition in 35–50% of all head and neck cancer patients, particularly in those with squamous cell carcinoma of the oropharyngeal and hypopharyngeal areas (1–6).

Plasma arginine concentrations are reduced in patients with cancer, which indicates that arginine metabolism is disturbed in the presence of a malignancy (7–10). Arginine-enriched nutrition in postoperative head and neck cancer patients is known to improve short-term outcomes, such as local wound complications, fistula rates, and length of hospital stays (11, 12). Moreover, arginine supplementation is believed to augment specific and nonspecific antitumor mechanisms, such as retarding tumor growth and prolonging survival (13), whereby normal T lymphocyte function is essential because of its substrate arginine (10). Actual prevention of malignant progression only manifests in the initiation and promotion phases. With the appearance of arginase, decreased T cell proliferation, low expression of specific T cell receptors, and decreased production of cytokines have been observed (14). Myeloid-derived suppressor cells appear to play a key role in these mechanisms by infiltrating the tumor and producing arginase, which enables the tumor to develop a cover shield against immune attacks (15).

In addition, arginine might play a role in carcinogenesis via its enzymatic conversion by nitric oxide synthase (NOS) with concomitant formation of nitric oxide (NO). Long-term exposure to low levels of NO induced by chronic inflammation may promote carcinogenesis by inhibiting apoptosis (8, 16). In contrast, high levels of NO can be toxic for malignant cells (16, 17). These tumor-toxic mechanisms particularly play a role in the initiation and promotion phases of the carcinogenesis. Generally, arginine and arginine-derived NO participate in many overlapping and conflicting regulatory processes, which lead to cancer development and prevention.

Our hypothesis is that, after surgical removal of malignant tumors, remnant cells can proliferate. This stage is comparable with the initiation and promotion phases of carcinogenesis. In these circumstances arginine supplementation may improve T cell function and promote NO production. The combination of

1 From the Departments of Surgery (NB, MARV, and PAMvL), Nutrition and Dietetics and Internal Medicine (MAEvB-dvdS and JAEL), Otolaryngology/Head and Neck Surgery (CRL), and the Department of Epidemiology and Biostatistics (DIJK), VU University Medical Center, MB Amsterdam, Netherlands.
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3 NB and MAEvB-dvdS share first authorship.
4 Supported by Nutricia Nederland BV.
5 Address correspondence to PAM van Leeuwen, Department of Surgery, VU University Medical Center, Postbus 7057, 1007 MB Amsterdam, Netherlands. E-mail: pam.leeuwen@vumc.nl.

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these body defense mechanisms may combat remnant malignant cells. Thus, to prevent recurrence, arginine should be ideally administered perioperatively.

Until now, the long-term effects of arginine have never been explored. Therefore, the present study was designed to analyze the long-term (>10 y) survival of malnourished patients undergong head and neck surgery who received either arginine-enriched tube feeding or standard tube feeding. In this patient population, we studied the long-term overall survival, the long-term disease-specific survival, locoregional recurrence, distant metastases, second primary tumors, and the (possible) cause of death of head and neck cancer patients.

SUBJECTS AND METHODS

Patients

In the period 1994–1997, 56 patients undergoing surgery for head and neck cancer who entered the Department of Otolaryngology/Head and Neck Surgery of the VU University Medical Center, Amsterdam, were studied. Patients who were severely malnourished, defined as a preoperative weight loss ≥10% over the past 6 mo, were eligible for inclusion (18). The study was a prospective, randomized, double-blind, controlled clinical trial. All patients had a diagnosis of histologically proven squamous cell carcinoma of the oral cavity, larynx, oropharynx, or hypopharynx (18). Patients were excluded from the study if they received other investigational drugs or steroids; had renal insufficiency, hepatic failure, or any genetic immune disorder; or had a confirmed diagnosis of AIDS (18). The study was approved by the medical ethics committee of the VU University Medical Center (18).

This long-term survival study limited itself to 2 subgroups of patients: those who received standard enteral nutrition (control group) and those who received the arginine-enriched nutrition (arginine group) both preoperatively and postoperatively. The products were produced and blinded in the Netherlands (Nutricia Laboratories). An independent statistician generated the blinding procedure.

Nutrition

After stratification for type of surgery (combined mandibular resection or total laryngectomy) and previous radiotherapy (yes or no), the patients were randomly assigned to the arginine group or to the control group. The arginine group (n = 17) received preoperative and postoperative enteral nutrition in which 41% of the casein was replaced by arginine, whereas the control group (n = 15) received preoperative and postoperative enteral nutrition with a specifically formulated product that closely reflected the current standard of practice (standard formula) (Table 1). Nutritional solutions were isocaloric and isonitrogenous. The only difference between the 2 was the amount of arginine. Specifically, no other immune-modulating nutrients (eg, omega-3 fatty acids, nucleotides, and antioxidants) were added.

Target intake was based on estimated energy requirements, calculated as 1.5 × the basal energy expenditure based on actual body weight. The patients were given their complete nutritional requirements by enteral feeding, but they were allowed to eat in addition to tube feeding on demand.

Postoperatively, all patients received the same tube feeding (1.5 × basal energy expenditure), either the arginine or the control product, starting on the first postoperative day until an X-ray conducted to assess swallowing ability performed 10 d after surgery showed no leakage from anastomoses. (Repeated “swallowing X-rays” were scheduled if anastomotic leakage occurred.) Patients were not allowed to eat next to their tube-feeding until 10 d after surgery. If patients were in need of prolonged tube-feeding after the 10th postoperative day, they were all given standard tube-feeding (ie, the special formula was not continued).

Event monitoring

The data were collected in August 2007, after a follow-up period of ≥10 y. The following events were monitored: survival or death, the occurrence of locoregional recurrence, the occurrence of distant metastases, and the occurrence of second primary tumors. The cause of death was categorized as follows: 0, alive; 1, in-hospital-death; 2, death from recurrent cancer; 3, death from second primary tumor; and 4, death other than cancer related. Causes 1, 2, and 3 were noted as disease-specific causes of death. Survival analyses were made for both overall survival and disease-specific survival. The variables were expressed in months after the date of surgery.

Statistical procedures

Curves for overall survival, disease-specific survival, and disease-free survival were made according to the Kaplan-Meier method. Log-rank tests were used to compare survival between the control group and the arginine group. Cox regression was used to study confounding and effect modification. To bring the effect of arginine supplementation into perspective, crude hazard ratios (HRs) were computed with Cox regression and tabulated for the grouping variable and for several well-known risk factors. Calculations were made with SPSS version 16.0 (SPSS Inc, Chicago, IL) computer software. All P values <0.05 were considered to indicate statistical significance.

| TABLE 1 |
| Composition per liter of the nutritional formulas/ |
| Supplement |
| Standard formula | Arginine formula |
| Protein (g) | 62.50 | 36.85 |
| Free arginine (g) | 0.00 | 12.50 |
| Glutamine (g) | 6.30 | 3.70 |
| Nitrogen (g) | 9.80 | 9.80 |
| Fat (g) | 48.61 | 48.61 |
| Carbohydrate (g) | 140.63 | 153.77 |
| Energy (kJ) | 5250 | 5250 |

Assumptions: 1 g protein = 17 kJ, 1 g arginine = 17 kJ, 1 g carbohydrate = 17 kJ, 1 g fat = 38 kJ, glutamine content = 10% by weight of casein, conversion factor for casein = 6.38, and arginine contains 32.16% nitrogen.
RESULTS

Patients

Seventeen patients were randomly assigned to the arginine group and 15 patients to the control group. None of the patients dropped out after randomization. Preoperatively, all 32 patients were given enteral nutrition for 7–10 d through a nasogastric feeding tube. The groups did not differ significantly in age, sex, tumor stage, tumor localization, comorbidity, weight loss, type of operation, or type of reconstructive surgery (Table 2). In the preoperative period, patients in the arginine group reached 113% and patients in the control group 110% of their estimated energy requirements, with 15% and 10% of intake originating from oral food intake, respectively. Preoperative tube feeding was provided for 8.6 ± 1.4 d (arginine group) and 8.8 ± 1.4 d (control group). Postoperatively, patients in the control group reached 96 ± 9% of their estimated requirements, whereas patients in the arginine group reached 83 ± 18% of their estimated requirements (NS). Overall, no difference in energy and protein intakes was observed between the groups, neither preoperatively nor postoperatively. For the patients in the arginine group, the mean total arginine intake over the preoperative and postoperative periods added up to 1015 ± 307 g—ie, 50 ± 15 g/d or 0.72 ± 0.19 g · kg⁻¹ · d⁻¹.

Long-term survival

All 15 patients in the control group and 14 of 17 patients in the arginine group had died at the time of data collection. Sixteen patients had died of the consequences of recurrent cancer (locoregional recurrence and/or distant metastases), and 4 patients had died because of a second primary tumor. Three patients died within the first 30 d after surgery (in-hospital death), and 6 patients died of causes other than cancer and were censored from the disease-specific survival analyses.

The median overall long-term survival was 34.8 mo in the arginine-supplemented group and 20.7 mo in the control group (P = 0.019; Figure 1). Disease-specific survival was 94.4 mo in the arginine-supplemented group and 20.8 mo in the control group (P = 0.022).

To exclude the influence of well-known, prognostic factors (TNM stage, margins, lymph nodes, preoperative weight loss, sex, and age) on survival, Cox regression analyses were performed to test for confounding and effect modification. N stage, and to a lesser extent weight loss and T stage, turned out to be confounders. When the confounders (either individually or combined) were entered into the model, the differences in survival between the arginine and control groups remained significant (P = 0.031 with all 3 confounders in the model).

To put the effect of arginine supplementation into perspective, crude HRs were computed with Cox regression and tabulated for the grouping variable and for several well-known risk factors. The results for overall survival are shown in Table 3. Similar results were found for disease-free survival (not depicted in the table).

Being assigned to the arginine group had a positive influence on both overall survival (HR: 2.632; 95% CI: 1.142, 6.061) and disease-free survival (HR: 4.167; 95% CI: 1.389, 12.500). Absence of extracapsular spread had a positive effect on disease-free survival (HR: 2.8120; 95% CI: 1.008, 7.831) but not on overall survival. Lower tumor stages had marginally significant effects on overall survival, but these results attenuated disease-free survival. In the first instance, age was entered as a linear term in the model. However, because of the possibility on nonlinear effects, age was also entered as a nominal variable and a time-dependent covariate, which did not change the results.

Locoregional recurrence

Locoregional recurrence occurred in 4 of the 17 patients in the arginine group and in 9 of the 15 patients in the control group. Because less than half of the patients in the arginine group had died of locoregional recurrence, median time until development of locoregional recurrence could not be estimated, but was >92.8 mo; it was estimated at 10.6 mo in the control group (P = 0.027; Figure 2).
TABLE 3
Hazard ratios (HRs) for the influence of perioperative variables on survival

<table>
<thead>
<tr>
<th>Perioperative variables</th>
<th>P</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (arginine/control)</td>
<td>0.023</td>
<td>2.632</td>
<td>1.142, 6.061</td>
</tr>
<tr>
<td>Age</td>
<td>0.155</td>
<td>0.975</td>
<td>0.942, 1.010</td>
</tr>
<tr>
<td>Weight loss (%)</td>
<td>0.188</td>
<td>1.047</td>
<td>0.978, 1.120</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>0.245</td>
<td>0.639</td>
<td>0.301, 1.360</td>
</tr>
<tr>
<td>Extracapsular spread (no/yes)</td>
<td>0.293</td>
<td>1.539</td>
<td>0.689, 3.440</td>
</tr>
<tr>
<td>Major postoperative complications (no/yes)</td>
<td>0.139</td>
<td>1.757</td>
<td>0.833, 3.705</td>
</tr>
<tr>
<td>Radical surgery (no/yes)</td>
<td>0.513</td>
<td>1.285</td>
<td>0.607, 2.270</td>
</tr>
<tr>
<td>N stage (≥N2)</td>
<td>0.552</td>
<td>1.256</td>
<td>0.593, 2.660</td>
</tr>
<tr>
<td>T stage (≥T4)</td>
<td>0.064</td>
<td>2.452</td>
<td>0.949, 6.336</td>
</tr>
<tr>
<td>Stage (≥IVa)</td>
<td>0.086</td>
<td>2.934</td>
<td>0.858, 10.037</td>
</tr>
</tbody>
</table>

All patients with locoregional recurrence had died at the time of analysis; 9 of them were reported to have locoregional recurrence only, whereas another 4 were reported to have locoregional recurrence in combination with distant metastases. Locoregional recurrence-specific survival differed significantly between the 2 groups (P = 0.010). When the confounders were accounted for, the observed effect became even more pronounced.

Distant metastases and second primary tumors

We observed no significant differences between either the development of distant metastases or the occurrence of second primary tumors between the arginine-supplemented and the control groups.

DISCUSSION

To our knowledge, our study was the first to show that a nutritional intervention with arginine-enriched nutrition before and after surgery may improve survival. Overall survival was a median of 34.8 mo in the arginine group and 20.7 mo in the control group; disease-specific survival was a median of 94.4 and 20.8 mo, respectively.

Despite the available surgical treatments, the survival of head and neck cancer patients remains disappointing because of uncontrollable persistent and recurrent disease. The most important prognostic factors are histologic tumor grade, clinical stage, tumor invasion, tumor thickness, angio-lymphatic invasion, and dissemination (19, 20). Within our study, pathologic results concerning malignant tissue removed from the patients showed no significant differences in tumor stage and localization between groups. Furthermore, the type of resection and the type of reconstructive surgery were not significantly different between the 2 groups. Although suggested as prognostically important, the true predictable value is controversial.

Generally, in patients with cancer, immune suppression can be caused by malnutrition, surgical trauma, and the immunosuppressive capacity of the tumor itself. The immune suppression in these patients can be linked to low levels of arginine (15, 18, 21). Nutritional support with arginine is suggested to improve nutritional status and immune function, which may subsequently result in enhanced defense against tumor growth (22–25). Although the mechanisms by which our positive results can be explained remain speculative, interplay of arginine and immunologic defense seems plausible. Our hypothesis, advocating the concept of proliferating remnant malignant cells that could be attacked by arginine-induced improved T cell function and enhanced NO production, originates from several pioneering studies (10, 15, 26). This hypothesis might also explain the difference in short-term survival and long-term outcome. Besides the large influence of direct postoperative effects, the primary arginine effect occurred during the supplementation phase, but the result was observed much later. First, the effects of arginine on NO metabolism have been observed in animal and human studies (27). Furthermore, increased concentrations of NO in the microenvironment have been noticed to induce cytostasis and cytotoxicity in tumor cells and to induce apoptosis by stimulating the tumor suppressor gene p53 (16, 17, 28, 29). Recent data support the importance for this latter specific action. In surgical patients with head and neck squamous cell carcinoma, some patients were shown to still possess p53 mutations in adjacent epithelial cells (30–32). Most head and neck squamous cell neoplasms are observed to develop within a field of premalignant cells, which show alterations associated with the process of carcinogenesis involving mutations in the p53 genes. Other recent studies provide additional similar evidence. After surgical cancer resection, part of the “field” may be left in the patient (33).

Thus, arginine-derived NO could have activated the intact p53 genes and thereby switched on the immune system to clear this tissue from the residual normal-appearing cells (28, 29). This may have been one of the key principles to explain better survival and lower the appearance of locoregional recurrence and longer recurrence-free survival in the arginine-supplemented group.

Second, arginine is essential for normal T cell proliferation. T cells play an important role in the body’s defense against malignant cells. Rodriguez et al (14) showed decreased proliferation, a low expression of T cell receptor CD3zeta chain, and a decreased production of cytokines in appearance of arginase—the enzyme that converts arginine. Moreover, high arginase activity surrounding the tumor has been suggested to host arginine metabolism, which subsequently leads to reduced arginine...
concentrations in cancer patients. These reduced arginine concentrations are observed independently of tumor type, tumor stage, and body mass index as investigated by Vissers et al (9).

Furthermore, arginase is produced by myeloid-derived suppressor (MDS) cells (described as macrophages or immature dendritic cells) that are observed to deplete arginine and to impair T cell proliferation by infiltrating the tumor (10, 26, 34). Different types of these myeloid cells are already detected in colon, lung, and renal cell tumors and could be well present in many other types of cancer (10, 15, 34). Interestingly, blocking arginase eliminated the suppressor function of the MDS cells in vitro and even showed antitumor effects in vivo, because of normalized arginine metabolism. Arginine depletion could thus be a mechanism by which the tumor regulates the immune system in favor of its own survival. Therefore, immunotherapy could be of great value to restrict this mechanism, which could be accomplished by arginase blocking therapy or arginine administration and thereby improve arginine balance (10, 35). However, no significant difference in the inflammatory response (lymphocytes, tumor necrosis factor-α, interleukin-6, and C-reactive protein concentrations) was observed in patients with head and neck cancer between an arginine-supplemented group and a control group (36).

Supplemental arginine has been administered in a variety of clinical trials. However, many studies have suggested that arginine can reduce tumor growth, and some have observed a negative influence on tumor growth. Nevertheless, the approach is controversial (37, 38). New data showed that arginine can be converted into glutamine (39). Future studies could be done with glutamine and possibly show an even more profound effect. On the basis of current knowledge, we advocate arginine supplementation in the initiation and promotion phases. Arginine should not be given in the subsequent (progression) phases of cancer, because it could stimulate angiogenesis and tumor growth through mechanisms involving NO and growth factors. A recent study by Ascierto et al (40) showed that reducing arginine concentrations in patients with metastatic melanoma may result in prolonged survival.

The benefit of arginine supplementation has been reported in several studies and has been registered in the European Society for Clinical Nutrition and Metabolism guidelines (41). Jones and Heyland (42) hypothesized that arginine supplementation could inhibit arginase and thus prevent dysfunction of the immune system. The significant better locoregional recurrence-free survival in the arginine group supports our hypothesis that arginine-derived NO and T cells can provide surveillance against (pre) malignant cells after surgery.

Arginine was also shown to block the formation and development of colorectal tumors, as observed in restrained crypt cell hyperproliferation and enhanced expression of survival (an inhibitor of apoptosis). This might be related to increased NO concentrations and decreased arginine conversion by arginase (43). These results strongly support our hypothesis and clinical results.

As a result of the conflicting effects of arginine on cancer, a review reported the following: "Long term data regarding the impact of arginine supplementation on mortality are not available. It had been suggested that this is probably a result of persistent concern about a possible promotion of tumor growth in some cases." (37). Because of the long-term follow-up period, we were able to investigate the long-term effects of perioperative enteral arginine supplementation in head and neck cancer patients. Our study is innovative because it involved single nutrient supplementation, homogeneous patient groups, and the length of follow-up.

The long-term effects of arginine supplementation on survival in the current study should be considered promising; still, small numbers of patients were included, and contemplations with respect to future studies are inevitable. Postoperative factors, such as lifestyle (which includes exercise, a reduction in alcohol, and healthy food) and malnutrition, may have influenced the results between the groups. However, this study was not powered and designed to adequately rule out these factors. Therefore, we cannot exclude that these confounding factors might have influenced the results.

However, this study inevitably suggests that arginine may be a potential new valuable player in the treatment of head and neck cancer patients: this apparently simple intervention is suggested to improve the prognosis of these patients. Larger studies are necessary to confirm our results and to disentangle the underlying mechanisms of the therapeutic activity of arginine on tumor cells and immune function.

In conclusion, this study suggests that arginine-enriched nutrition given perioperatively may be a valuable tool for improving long-term survival in malnourished head and neck cancer patients. Larger groups are needed to confirm these results.

The authors’ responsibilities were as follows—NB and MAEvBvdS: were responsible for the study design, data collection, and data analyses and drafted the manuscript; JAEJ: critically revised the manuscript and helped with data analyses; CRL: interpreted the data and critically revised the manuscript; DJK: responsible for the statistical analyses; MARV: interpreted the data and helped draft the manuscript; and PAMvL: responsible for all parts of the study and supervised the study. All authors read and approved the final manuscript. None of the authors had any conflicts of interest to disclose. Nutricia Nederland BV did not participate in the data-analysis, data-interpretation, or manuscript writing.

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