Plasma Osteopontin Levels in Patients With Head and Neck Cancer Undergoing Chemoradiotherapy

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Objectives: To explore the prognostic role of plasma levels of osteopontin (OPN), a phosphoglycoprotein with adhesive properties, in patients with head and neck squamous cell carcinoma (HNSCC) undergoing concomitant chemoradiotherapy. Previous studies have proposed OPN level as a prognostic factor in several cancers.

Design: Prospective analysis of plasma OPN levels, before and within 12 weeks after treatment, in a cohort of patients with HNSCC undergoing platinum-based chemoradiotherapy at our center.

Setting: Academic center.

Patients: Sixty-nine patients diagnosed as having HNSCC.

Interventions: Plasma levels of OPN were assessed before the start and after the conclusion of chemoradiotherapy by using an enzyme-linked immunosorbency assay kit. Chemoradiotherapy was exclusive (n = 52) or adjuvant to surgery (n = 17).

Main Outcome Measures: Levels of OPN were correlated with clinicopathological characteristics, response to treatment, and overall survival.

Results: Pretreatment plasma OPN levels were higher in patients with advanced T and N stages compared with patients with early stages (P = .009 and .07, respectively). Mean (SD) plasma levels of OPN measured before (102.5 [68.1] ng/mL) and after (104.0 [53.6] ng/mL) treatment did not differ (P = .18, paired t test). Pretreatment and posttreatment levels of OPN were lower in patients who achieved a complete response compared with those who failed to respond (75.0 [41.5] vs 131.2 [82.9] ng/mL [P = .005] and 86.8 [40.5] vs 141.6 [58.4] ng/mL [P = .004], respectively). Patients with high pretreatment OPN levels (>82.1 ng/mL) had shorter survival time (P < .001). Posttreatment OPN levels were marginally (P = .10) associated with survival time in univariate analysis.

Conclusions: In patients with HNSCC undergoing chemoradiotherapy, a low pretreatment plasma OPN level is associated with treatment response and better survival. Modulation of OPN levels by chemoradiotherapy may also be associated with outcome. Further studies with serial measurement of OPN levels are warranted in these patients.

Plasma OPN expression was described as an independent marker of shorter survival in a cohort of 140 HNSCC patients undergoing various treatment modalities. In a small series of Japanese HNSCC patients, higher serum levels of OPN were associated with advanced tumor stages. In the only published study on the interaction of OPN expression and the response to a radiosensitizer, high levels of OPN expression seemed to select for patients who benefited from treatment.

For these reasons, the aim of this study was to explore the prognostic role of plasma OPN levels in HNSCC patients undergoing concomitant chemoradiotherapy.

## Methods

### Study Population

Criteria for patients participating in the study included (1) the presence of histologically confirmed HNSCC, (2) no previous radiation treatment or chemotherapy, and (3) the indication, by the treating surgeon, for chemoradiotherapy as an exclusive treatment or as an adjuvant treatment after surgery. Exclusive chemoradiotherapy was indicated in patients with unresectable or medically inoperable tumors. Unresectability criteria included invasion of the base of the skull, the prevertebral fascia, the cervical spine, or the carotid artery. Adjuvant chemoradiotherapy was indicated in patients whose resected tumors showed any high-risk feature, defined as more than 2 compromised lymph nodes, extracapsular extension in compromised lymph nodes, and close or microscopically compromised tumor margins. In addition, patients had to be willing to sign an informed consent approved by the institutional review board of our institution, in accordance with the guidelines of good clinical practices and Brazilian law. From January 1, 2005, through December 31, 2006, 69 patients who fit these criteria were enrolled in the study, conducted at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. Tumor staging was performed according to the 1998 American Joint Committee on Cancer system, and treatment followed the routine guidelines of our institution. The intended treatment included radiotherapy of 70 Gy for exclusive therapy or 60 to 66 Gy for adjuvant therapy in daily 2-Gy fractions and with concomitant chemotherapy (cisplatin, 100 mg/m²) on days 1, 21, and 43. Treatment response evaluation was performed within weeks of treatment completion. A complete tumor response was defined as no tumor detection by means of clinical and imaging studies. Overall survival was defined as the interval from the start of chemoradiotherapy to death, with the last follow-up in February 2008.

### Sample Collection and OPN Plasma Assay

Blood samples were collected before the start of chemoradiotherapy (n=69) and within 12 weeks of treatment completion (n=46). Blood aliquots were withdrawn into tubes with EDTA anticoagulant, stored at 4°C for a maximum of 16 hours, and subsequently centrifuged at 3000 rpm for 15 minutes. The resulting plasma was divided into aliquots and stored at −70°C. We measured OPN levels using an enzyme-linked immunosorbent assay kit (IBL-Japan, Gunma, Japan), following the manufacturer’s instructions. Samples were tested in duplicate, and the results were determined by a standard curve, which was built using the human OPN control level supplied by the kit.

### Statistical Analysis

We used analysis of variance (ANOVA) to test for differences in OPN level across the various clinicopathological categories. A paired t-test was used to determine the significance of differences between pretreatment and posttreatment OPN levels. Linear regression and χ² tests were used when appropriate.

To test for factors associated with tumor response and survival, OPN values were characterized as OPN-negative or OPN-positive according to their relation to the median value. Survival analysis was performed using the Kaplan-Meier method, and differences between groups were assessed using the log-rank test. A P-value of less than .05 was considered significant.

### Results

A total of 69 patients were included in this study. Most were men, with a median age of 57 years and a history of tobacco and alcohol consumption. Mild anemia was present in most patients. Results of histopathological analysis confirmed the diagnosis of squamous cell carcinoma in all patients, most with a moderately differentiated grade. The most commonly affected site was the larynx, followed by the oral cavity and oropharynx.

Fifty-two patients were treated exclusively with chemoradiotherapy and 17 with surgery followed by adjuvant chemoradiotherapy. Median radiation and cisplatin doses were 70 Gy and 260 mg/m², respectively, delivered in a median of 58 (range, 49-90) days.

### OPN Level and Clinicopathological Characteristics

The mean (SD) plasma OPN concentration for all 69 patients before the start of chemoradiotherapy was 102.5 (68.1) ng/mL, with a median of 82.1 ng/mL and a range of 3.9 to 333.8 ng/mL. Correspondent posttreatment values in 46 patients were a mean (SD) concentration of 104.0 (53.6) ng/mL, a median of 92.9 ng/mL, and a range of 19.4 to 247.8 ng/mL. No difference was found between the OPN values before and after treatment (P=.18, paired t test) or between the subgroups undergoing exclusive or adjuvant (P=.19) chemoradiotherapy. When measured in individual patients, the magnitude of the difference between posttreatment and pretreatment OPN levels varied widely, from 157.7 to 128.6 ng/mL, with a median level of 11.7 ng/mL.

### Among clinicopathological variables, OPN levels were higher in patients with advanced T and N stages compared with patients with early stages (P=.009 and .07, ANOVA, respectively). Undifferentiated tumors were associated with numerically higher (although not statistically different) levels of OPN. The tumor site did not seem to influence OPN levels, although patients with tumors of the hypopharynx initially had numerically higher OPN levels compared with patients with other sites (P=.01). The tumor size did not influence OPN expression, although patients with tumors larger than 4 cm had numerically higher OPN levels (P=.04).

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OPN LEVEL VARIATION AND TUMOR RESPONSE

Of the 52 patients undergoing exclusive chemoradiotherapy, 22 (42%) had a complete response and 30 (58%) were classified as failing to respond to treatment. The latter were subclassified as having progressive disease (11 patients [21%]), stable disease (3 [6%]), and a partial response (16 [31%]). When measured before and after treatment, mean (SD) plasma OPN levels were lower in patients who achieved a complete response compared with patients who failed to respond (pretreatment levels, 75.0 [41.5] vs 131.2 [82.9] ng/mL [P = .005]; posttreatment levels, 86.8 [40.5] vs 141.6 [58.4] ng/mL [P = .004]) (Figure 1).

In the subgroup of patients undergoing exclusive chemoradiotherapy who had a complete response and both pretreatment and posttreatment OPN measurements available (17 of 22), the paired mean (SD) pretreatment and posttreatment OPN values did not differ (70.1 [44.2] and 86.8 [40.5] ng/mL, respectively [P = .14]), although, numerically, the OPN levels decreased in 7 patients and increased in 10.

Factors associated with a complete response, as determined by chi-square analysis, included T status (P = .007), N status (P = .002), pretreatment OPN level (categorized according to its relation to the median value of 82.1 ng/mL) (P = .007), posttreatment OPN level (categorized according to its relation to the median value of 92.9 ng/mL) (P = .02), and tumor site (categorized as oral cavity vs other site) (P = .02). Radiotherapy and chemotherapy doses and treatment duration were not associated with a complete response.

OPN LEVEL AND SURVIVAL

After a median follow-up of 23.5 (range, 8.0-37.0) months for living patients, 30 of 69 patients had died. In univariate analysis, the log-rank test showed that T and N stages and tumor site predicted overall survival (P = .01, .007, and .001, respectively). A pretreatment plasma OPN level above the median value for the whole group (82.1 ng/mL [an OPN-positive value]) was a negative predictor of overall survival in univariate analysis, with a median survival of 9.3 months, compared with patients with pretreatment OPN levels at or below the median value (82.1 ng/mL [an OPN-negative value] [P < .001, log-rank test]), with a total of 22 of 35 and 8 of 34 deaths, respectively. Posttreatment OPN levels, categorized according to their relation to the median value for the whole group (92.9 ng/mL), were marginally predictive of survival by the log-rank test (P = .10) (Figure 2).

COMMENT

In the present study, we evaluated plasma OPN levels in HNSCC patients before the start and after the comple-
tion of chemoradiotherapy. We did not find any statistically significant difference between the paired values or any association between response and OPN level changes in individual patients, perhaps owing to the wide range of values, small sample size, and evaluation at only 2 time points. Based on the hypothesis that serial measurements would possibly be more informative, only 2 studies, to our knowledge, have previously evaluated changes in plasma OPN levels over time. The first study reported data in patients with metastatic breast cancer and showed that increases in plasma OPN levels over time were strongly associated with a poor survival.9 In contrast, a study of multiple myeloma did not find any association.18

In our study, we found higher pretreatment plasma OPN levels in patients with advanced T and N stages compared with those with earlier stages, which is in agreement with previously published data in tongue cancer14 and in a cohort of 37 patients with laryngeal and hypopharyngeal tumors.16 In these studies, OPN levels were assessed by immunohistochemistry14 and in the patients’ serum samples,16 and higher values were also associated with high-risk pathological features.

We have demonstrated that high pretreatment plasma OPN levels were associated with a lack of response to chemoradiotherapy, which agrees with the results from another study, in which patients were treated exclusively with radiotherapy.17 In our patients, we found that OPN and hemoglobin levels were inversely correlated, which is in agreement with previous works reinforcing the link between OPN level and tissue hypoxia.17,19,20 In addition, lower posttreatment OPN levels were also associated with a complete tumor response, suggesting a link between tumor bulk and plasma OPN levels.

We were surprised, however, to find that OPN levels did not decrease in the small subgroup of responding patients who had pretreatment and posttreatment measurements. A possibility is that tumor destruction could cause a transient increase in plasma OPN levels, liberated by dying cells or tumor stroma. Because the timing of our posttreatment collection differed among patients, serial posttreatment OPN measurements would be useful to

![Figure 1](image1.png)  
**Figure 1.** Plasma osteopontin (OPN) levels in 52 patients undergoing exclusive chemoradiotherapy. Levels were measured before (A) and after (B) chemoradiotherapy and are categorized according to response status. Thirty-two patients undergoing exclusive chemoradiotherapy had postoperative OPN levels available for analysis. Yes indicates a complete response and no, a partial response or failure to respond. Boxes indicate the 25th and 75th percentiles; horizontal lines in each box, medians; whiskers, the minimum and maximum values, extending to 1.5 times the interquartile range; and circles, outliers.

![Figure 2](image2.png)  
**Figure 2.** Overall survival curves of patients with head and neck squamous cell carcinoma undergoing chemoradiotherapy, categorized according to plasma osteopontin (OPN) levels. The OPN values were categorized as positive or negative according to values above the median value or at or below the median value, respectively. Pretreatment OPN levels (A) were available in 69 patients (median level, 82.1 ng/mL); posttreatment levels (B) were available in 46 patients (median level, 92.9 ng/mL).
clarify the issue, as previously shown in breast cancer and multiple myeloma. Another possibility is the existence of residual disease in some patients with a complete response, as determined by conventional methods. In this situation, survival analysis may be a better indicator of treatment result.

In terms of survival, a pretreatment OPN level at or below the median (≤82.1 ng/mL) was associated, in univariate analysis, with a better overall survival. This is in agreement with previous data on HNSCC patients undergoing different treatment modalities. Posttreatment OPN levels, categorized according to their relation to the median level of 92.9 ng/mL, constituted only a marginally significant predictor of survival in univariate analysis, perhaps related to a reduced tumor mass but taking into consideration the confounding factors discussed in the preceding paragraph.

The limitations of our study warrant some comments. Distinct commercially available enzyme-linked immunosorbent assay kits yield different OPN values, which make it difficult to compare studies and define clinically useful cutoff values. In this regard, our absolute values are similar to the ones reported in the only study using the same kit in HNSCC patients. Although patients with missing OPN values did not differ from the whole group regarding T and N stages and tumor site (data not shown), missing values could have biased our results.

In summary, in this group of high-risk HNSCC patients, low pretreatment plasma OPN levels were associated with tumor response and better survival. In addition, modulation of OPN levels by means of chemoradiotherapy may also be associated with outcome. Thus, further work with serial measurements may be warranted in these patients.

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Author Contributions: Drs Snitcovsky, Pasini, Mangone, Maistro, and Federico and Ms Brunialti had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Snitcovsky and Federico. Acquisition of data: Snitcovsky, Leitao, Brunialti, de Castro, and Villar. Analysis and interpretation of data: Snitcovsky, Leitao, Pasini, Brunialti, Mangone, Maistro, and Federico. Drafting of the manuscript: Snitcovsky, Pasini, Brunialti, Mangone, Maistro, and Federico. Critical revision of the manuscript for important intellectual content: Snitcovsky, Leitao, Pasini, Mangone, Maistro, de Castro, Villar, and Federico. Statistical analysis: Snitcovsky. Obtained funding: Federico. Administrative, technical, and material support: Pasini, Brunialti, Mangone, Maistro, de Castro, Villar, and Federico. Study supervision: Snitcovsky, Leitao, and Federico.

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REFERENCES


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