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Perineural Invasion in Aggressive Skin Carcinomas of the Head and Neck

Potentially Dangerous but Frequently Overlooked

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Key Words

Perineural invasion · Basal cell carcinoma · Squamous cell carcinoma · Base of skull · Immunohistochemistry

Abstract

Introduction: Perineural invasion is a well-recognized form of cancer dissemination. However, it has been reported only in few papers concerning cutaneous carcinomas (basal cell, BCC, and squamous cell, SCC). Moreover, the incidence is considered to be very low. Niazi and Lambert [Br J Plast Surg 1993;46:156–157] reported only 0.18% of perineural invasion among 3,355 BCCs. It is associated with high-risk subtypes, as morphea-like, as well as with an increased risk of local recurrence. No paper was found in the literature looking for perineural invasion in very aggressive skin cancers with skull base extension, with immunohistochemical analysis. ***Methods:*** This is a retrospective review, including 35 very advanced skin carcinomas with skull base invasion (24 BCCs and 11 SCCs, operated on at a single institution from 1982 to 2000). Representative slides were immunohistochemically evaluated with antiprotein S-100, in order to enhance nerve fibers and to detect perineural invasion. The results were compared to 34 controls with tumors with a good outcome, treated in the same time frame at the same Institution. ***Results:*** Twelve (50.0%) of the BCCs with skull base invasion had proven perineural invasion, as opposed to only 1 (4.6%) of the controls, and this difference was statistically significant ($p < 0.001$). Regarding SCCs, 7 aggressive tumors (63.6%) showed perineural invasion compared to only 1 (10.0%) of the controls, but this difference did not reach significance ($p = 0.08$), due to the small number of cases. ***Conclusions:*** In this series, it was demonstrated that immunohistochemically detected perineural invasion was very prevalent in advanced skin carcinomas. In addition, it was statistically associated with extremely aggressive BCCs with skull base invasion.

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Introduction

Skin carcinomas are the most prevalent human malignant tumors, basal cell carcinoma (BCC) being more frequent than squamous cell carcinoma (SCC) [1]. The incidence is particularly high among Caucasians after the sixth decade, who suffered chronic sunlight exposure [2]. Occasionally, skin cancers may be exceptionally aggressive, invading deeper anatomical planes and destroying soft tissues, bone and even the dura mater and brain. Some authors have recently reported their experience with skin carcinomas with skull base involvement [3]. In some published Brazilian skull base surgery series, they represent the main histological types of tumor [4–6].

Several factors have been associated with increased aggressiveness of these tumors: histopathological subtype (morphea-like and metatypical are considered aggressive subtypes), differentiation, depth of invasion and perineural invasion, among many others [7]. Perineural invasion has been considered an ominous prognostic factor in skin carcinomas [3, 8–12], especially when this invasion was detected by clinical and/or radiological findings [11, 13, 14].

What is the true incidence of perineural invasion among skin carcinomas? Considering large published series that encompass all 'ordinary' BCCs, it seems rather uncommon, oscillating from 0.19% [15] to 0.49% [16]. Some authors reported an increased frequency of 3.8% in cancers treated by Mohs' micrographic surgery [17]. Others found a direct relationship between perineural invasion and aggressive BCC subtypes [18–20]. Goepfert et al. [21] observed a prevalence of 14% in 520 skin SCC of the head and neck, and these patients exhibited not only more local recurrences but also regional and distant metastases. All these authors used routine histopathological techniques, as hematoxylin-eosin (H&E) staining. However, it is possible to employ immunohistochemical analysis to improve the detection of nerve fibers. In fact, Schmitt and Bacchi [22] demonstrated the usefulness of immunohistochemical detection of S-100 protein to enhance nerve fibers. Recently, Lewis Kelso et al. [23] confirmed the superiority of this technique over regular H&E and described a new immunostaining method for discovering nerve fibers utilizing p75NGFR, a nerve growth factor receptor.

The objective of this study was to analyze the frequency of perineural invasion, using immunohistochemical detection of S-100 protein to facilitate the identification of peritumoral nerve fibers, in a consecutive series of very aggressive skin carcinomas with skull base invasion submitted to craniofacial oncological operations. In addition, these findings were compared to skin carcinomas with good outcome, treated in the same institution within the same time frame, in a case-control study.

Subjects and Methods

Patients

In this retrospective study, charts of patients with extremely aggressive BCC or SCC with skull base involvement treated at the Department of Head and Neck Surgery of the University of São Paulo Medical School were reviewed. Only cases with enough paraffin-embedded blocks to harvest slides for immunohistochemistry were included. Thirty-five patients constituted the 2 study groups: group 1 comprised 24 BCCs and group 2 included 11 SCCs. Seventeen patients (70.8%) in group 1 and 5 (45.5%) in group 2 had recurrent tumors, after surgery and/or radiotherapy. One patient (4.17%) in group 1, and 4 (36.36%) in group 2 had lymph node metastases. Four hundred and twenty-three charts were retrospectively analyzed, in order to create 2 control groups including patients with BCCs and SCCs located in the head and neck area, treated at the Dermatology Department of the University of São Paulo Medical School within the same time frame, with no recurrence for a minimum follow-up of 24 months (median follow-up: 36.3 months): group 3 comprised 23 BCCs and group 4 included 10 SCCs.

Immunohistochemical Analysis

The procedure described by Hsu et al. [24] in 1981 was employed. Representative slides obtained from the tumors were washed with a buffered saline solution at pH 7.4. They were then incubated in a buffered citrate solution at pH 6.4 for 15 min (Gown et al. [25]). Then, the slides were incubated with specific primary antibody against S-100 protein, diluted 1:300, for 12 h, at a temperature of 4°C. After washing with buffered saline solution, the slides were incubated for 60 min with biotinylated antibody anti-IgG (Vector Corp., USA). Then, they were incubated for 45 min with ABC Elite© complex (Vector Corp.). Finally, the slides were treated with 3.3′-diaminodibenzidine (Sigma Chemical Company, USA) and with peroxide 0.1% (Sigma Chemical Company). Counterstaining was performed with Harris hematoxylin for 5 min. The immunohistochemical built-in positive control was malignant melanoma cells.

Criteria for Interpretation of Immunohistochemistry Staining for Perineural Invasion with S-100 Protein

Immunoreactivity was considered positive when both cytoplasmic and nuclear Schwann cell staining were obtained. With this immunohistochemical enhancement, nerve fibers adjacent to the carcinoma were found to be normal (fig. 1) or destroyed (fig. 2), either partially or totally, by tumor infiltration. When no nerve fibers were evidenced near the cancer area, the result was considered inconclusive. All slides were blindly graded by 2 coauthors (A.F.L., C.E.B.), with good correlation scores between them. All cases were also submitted to routine H&E staining, and the results were blindly compared by the same 2 coauthors.

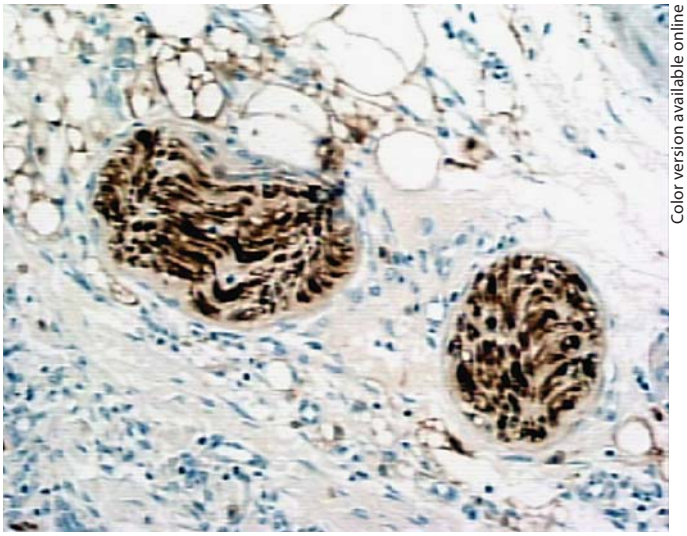

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Fig. 1. Immunostaining for protein S-100 showing an intact nerve. ABC technique; counterstaining with Harris hematoxylin. Original magnification. ×400.

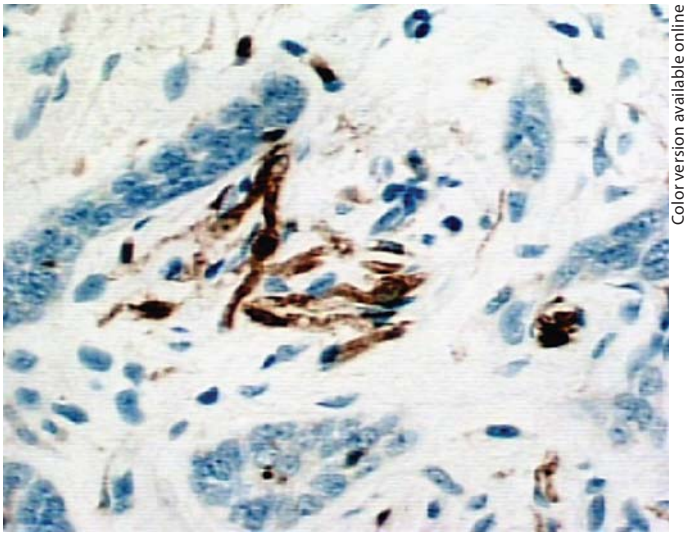

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Fig. 2. Immunostaining for protein S-100 showing a nerve with its fibers almost completely destroyed by cancer cells. ABC technique; counterstaining with Harris hematoxylin. Original magnification. ×400.

Statistical Analysis

For the statistical analysis, the Wald test, based on a linear model for categorized data (Agresti, 1990 [26]), was employed, to a significance level of 0.05.

Approval by Institution Internal Research Board

This study was officially approved by the internal research board of the Hospital das Clínicas of the University of São Paulo Medical School.

Results

Perineural Invasion in BCCs

Perineural invasion was found in 12 (50.0%) of the BCCs of group 1 (fig. 3). In contrast, only 1 BCC of group 3 (4.6%) had this invasion, and the difference was statistically significant ($p < 0.001$). Comparing the histological subtypes, considering all 47 BCCs of the 2 study groups, perineural invasion was more prevalent among patients with the morphea-like subtype, and this difference was also statistically significant (table 1). The routine H&E staining showed a reduced frequency of perineural invasion as opposed to the results with immunohistochemistry for protein S-100: 38.2 and 2.4% of the cases in groups 1 and 3 had detectable perineural invasion, compared to 50.0 and 4.6%, respectively, but this difference did not reach statistical significance ($p = 0.138$).

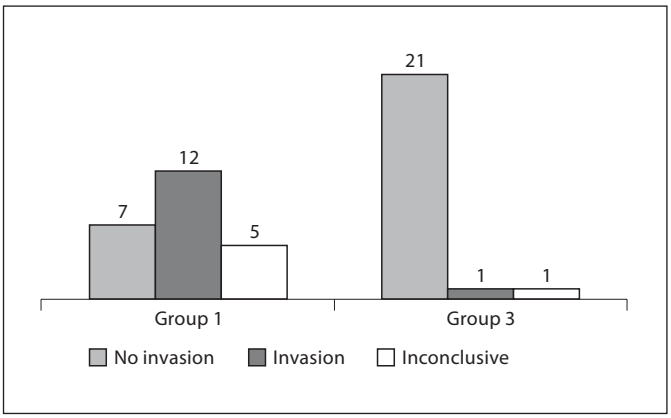

Fig. 3. Perineural invasion results in BCC patients (groups 1 and 3). $p < 0.001$.

Table 1. Histological subtypes

Histological subtype	Perineural invasion
Metatypical	5/8 (62.50)
Morphea-like	5/13 (38.46)
Solid	2/19 (10.53)
Adenoid	0/5

Figures are numbers of cases with percentages in parentheses. $p = 0.0031$.

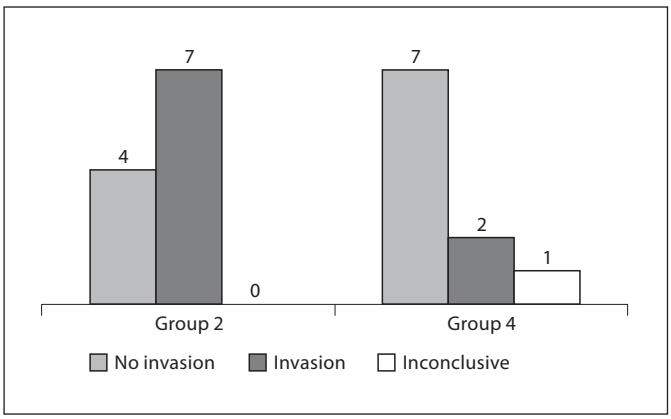

Fig. 4. Perineural invasion results in SCC patients (groups 2 and 4). p = 0.08.

Perineural Invasion in SCCs

Seven (63.6%) of the aggressive SCCs of group 2 showed perineural invasion, compared to only 1 (10.0%) of the tumors in group 4 (fig. 4). This difference, however, did not reach statistical significance (p = 0.08), probably due to the small number of cases. Perineural invasion was found at H&E staining in only 27.2% and 0 of the surgical specimens, again, with no statistically significant difference with immunohistochemistry (p = 0.091).

Discussion

Some skin carcinomas of the head and neck may have an extremely aggressive behavior, invading deeper anatomical planes and destroying soft tissues, bony structures of the face and skull and even the dura mater and/or brain. Evidently, the only surgical option in this situation is a combined craniofacial approach [3–6].

The formidable aggressiveness of some of these lesions has been associated with certain clinical and histopathological features [7]. In addition, some molecular and biological prognostic factors have also been identified [27–29].

Several authors have reported a clear association between perineural invasion and worse prognosis, generally due to increased recurrence rates [3, 8–12]. Backous et al. [3] described a series of 35 patients with nonmelanoma skin cancers submitted to oncological craniofacial resections; perineural invasion was one of the significantly negative prognostic factors (p = 0.049). Martin et al. [9] also identified perineural invasion as a statistically significant prognostic factor in a series of 31 metatypical skin cancers. Clayman et al. [12] studied 210 patients with skin SCCs. According to their results, perineural invasion was significantly linked with reduced disease-free survival (p = 0.002). Some authors have pointed out that overt clinical and/or radiological evident nerve invasion could worsen the patient's outcome even further [11, 13, 14].

How frequent is perineural invasion in skin carcinomas? The available data vary considerably, depending upon the subtype of tumor and methodology. Analysis of large series including all types of BCC suggested that this invasion is quite uncommon, ranging from 0.19% [15] to 0.49% [16]. When a more comprehensive microscopic search was performed, using Mohs' micrographic technique, perineural invasion was more frequent, as demonstrated by Ratner et al. [17]. They prospectively evaluated 434 patients submitted to Mohs' excision of BCCs, detecting perineural invasion in 3.8% of 78 cases that required more than 1 phase for its entire resection. Interestingly, they also found 'perineural inflammation' in 26.9% of these 78 cases. It is noteworthy that all these authors used just routine H&E staining, without any attempt to highlight nerve fibers. Yet, immunohistochemical staining is able to improve the detection of nerve fibers. As a matter of fact, Schmitt and Bacchi [22] demonstrated the usefulness of immunohistochemical detection of S-100 protein to enhance nerve fibers. This observation was confirmed by Lewis Kelso et al. [23], who also reported a new immunostaining method for enhancing nerve fibers with p75NGFR.

In the present series, perineural invasion was immunohistochemically detected in a significantly higher proportion both in BCCs and in SCCs. Regarding BCCs, 50.0% of the group 1 tumors had perineural invasion, and this percentage was far superior not only to the group 3 cases, but to all above-mentioned series as well. Interestingly, even the 4.6% positive invasion (which was really only 1 case) was superior to available reports. The aggressive subtypes showed an elevated prevalence of perineural invasion: nearly two thirds of the morphea-like and one third of the metatypical tumors. It is remarkable that these results were also considerably higher than previously reported series focusing on aggressive subtypes of BCC. Brown and Perry [18] analyzed 507 patients with morphea-like BCCs, observing a frequency of 3.0%. Leibovitch et al. [19] found a frequency of 7.9% of this invasion in 178 metatypical BCCs. The same group also reported a statistically positive association with morphea-like tumors [20]. Concerning the SCCs with skull base

invasion of group 2, the prevalence of perineural invasion was more than 6 times higher than in the control group 4, but this difference did not reach significance, due to the small sample size. Also, the prevalence of 63.6% was clearly superior to the 14.0% reported by Goepfert et al. [21].

This series of horrifying skin carcinomas with skull base invasion has been analyzed using immunohistochemical detection of other biological factors as well [27–29]. The control groups 2 and 4 were created using a computer analysis of 423 charts of patients with BCCs and SCCs that were treated at the Department of Dermatology of our institution during the same time frame, selecting the cases with a good outcome (no recurrence for at least 24 months) with better matching regarding epidemiological and staging features with the respective study groups. Some statistical comparisons among the groups were (between groups 1 and 3, for example): age: $p = 0.2264$; skin color: $p = 1.0000$; location: $p = 0.095$; length of follow-up: $p = 0.2767$. On the other hand, the T stage was clearly different ($p < 0.001$). We evaluated the 4 groups using 26 different epidemiological, clinical, histopathological and molecular factors, including: length of medical history, TNM staging, previous treatment, pattern of tumor infiltration, nuclear pleomorphism, inflammatory infiltrate, as well as dural and brain invasion, among others. Evidently, it is impossible to include all these data in a single manuscript. In addition, due to the retrospective nature of the study and to the obviously limited number of cases of such unusually invasive skin cancers, our honest aim with this and the other related and already published papers is to stimulate the appearance of other series of similar studies that could help detect, maybe in a multi-institutional way, sound prognostic factors and, eventually, correspondent molecular treatments.

In conclusion, the prevalence of perineural invasion employing immunohistochemical staining for S-100 protein in a population of extremely aggressive skin carcinomas with skull base invasion was significantly higher than in the control groups with tumors with a good outcome. Similarly, this prevalence was also much elevated when compared with previous series utilizing conventional H&E staining, suggesting a potential value for this methodology. Therefore, this study indicates that S-100 staining may increase the detection of perineural invasion, but due to the limited sample size and retrospective study limitations the exact clinical significance of this needs to be confirmed.

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