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Perineural Invasion in Oral Cancer: Clinical meaning and prognostic value

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Abstract

Cancer is currently one of the most common causes of morbidity and mortality, with more than 10 million new cases and 6 million deaths a year. Oral cancer is the eighth of the most diagnosed cancers worldwide, being squamous cell carcinoma responsible for more than 90% of the cases. Oral cancer is the common designation of the subtype of head and neck tumours that can occur in various locations such as lips, tongue, salivary glands, buccal floor and oral mucosa. It is usually very aggressive and difficult to treat and it usually has a reserved prognosis mainly because the diagnosis is made at an advanced stage of the disease. In spite of therapeutic advances, both of surgical therapies and radiotherapy and chemotherapy, the survival rate to 5 years has not changed significantly over the past decades and it is approximately 50%, since in advanced stages of the disease the different therapeutic modalities are ineffective. Therefore, efforts in the research and identification of new therapeutic strategies, as well as the research in order to identify new targets that allow early diagnosis and risk and prognosis factors of this pathology are justified. Perineural invasion (PNI) is one of the factors which, according to the literature, may influence the prognosis and increase the selection of patients who require more aggressive therapy.

The aim of this article is to carry out a review of the literature concerning the perineural invasion (PNI) in oral cancer and its clinical significance and prognostic value.

It was concluded that the perineural invasion occurs in various human cancers and has long been regarded as a prognostic factor independent of other factors, being regarded as a histological marker of aggressive disease and that may help identify patients with worse clinical course. It can also add important information to classic prognostic parameters. Due to the impact PNI may have on the prognosis and therapeutic decisions its histopathological observation must be part of the routine.

PNI, for which there is not a consensus definition yet, is an entity still barely studied and understood with a few contradictions as to its meaning prognosis, which makes its

study and understanding a challenge. More studies are needed and with them a better understanding of the mechanisms involved in the PNI.

A better knowledge of this entity and later diagnosis of its presence will be an asset enabling the maximization of the therapeutic success and the decrease of the morbidity and mortality associated with oral cancer.

Finally it is important never to forget the (importance) significance of early diagnosis of oral cancer. Delays in the identification and recognition of suspicious lesions are responsible for the delay of diagnosis and the advanced stage of the disease at diagnosis with very low survival rates.

Keywords:

Oral Squamous cell carcinoma; Perineural invasion in oral cancer; Perineural invasion in oral squamous cell carcinoma.

Resumo

O cancro é actualmente uma das causas mais comuns de morbilidade e mortalidade, com mais de 10 milhões de novos casos e 6 milhões de mortes por ano. O Cancro oral encontra-se na oitava posição dos cancros mais diagnosticados a nível mundial sendo carcinoma espinhocelular responsável por mais de 90% dos casos. É a designação comum do subtipo de tumores da cabeça e pescoço que pode ocorrer em várias localizações como os lábios, a língua, as glândulas salivares, o pavimento bucal e a mucosa oral. É geralmente muito agressivo e difícil de tratar com prognóstico geralmente reservado, principalmente porque o diagnóstico é feito em fase avançada da doença. Apesar dos avanços terapêuticos, tanto das terapêuticas cirúrgicas como da radioterapia e da quimioterapia, a taxa de sobrevivência, aos 5 anos não se alterou significativamente nas últimas décadas e é de aproximadamente 50%, já que nos estádios avançados da doença as diferentes modalidades terapêuticas são pouco eficazes. Justificam-se, assim, os esforços na pesquisa e identificação de novas estratégias terapêuticas bem como a investigação no sentido de identificar novos alvos que permitam o diagnóstico precoce e factores de risco e de prognóstico desta patologia. A invasão perineural é um destes factores que, segundo a literatura, pode influenciar o prognóstico e potenciar a selecção de doentes que necessitem de terapêuticas mais agressivas.

O objectivo deste artigo é efectuar uma revisão da literatura relativamente à invasão perineural (PNI) no cancro oral e o seu significado clínico e valor prognóstico.

Concluímos que a invasão perineural ocorre em vários cancros humanos e tem sido considerada como um factor de prognóstico independente de outros factores, sendo

considerada como um marcador histológico de doença agressiva e que poderá ajudar a identificar os doentes com pior evolução clínica, e pode adicionar informações importantes aos parâmetros prognósticos clássicos. Devido ao impacto que a PNI poderá ter no prognóstico e decisões terapêuticas a sua observação anatomopatológica deve fazer parte da rotina.

A PNI, para a qual ainda não existe uma definição consensual, é uma entidade ainda mal estudada e compreendida havendo algumas contradições quanto ao seu significado prognóstico, o que torna o seu estudo e compreensão um desafio.

Mais estudos são necessários e com eles um melhor conhecimento dos mecanismos envolvidos na PNI.

O melhor conhecimento desta entidade e posterior diagnóstico da sua presença será uma mais valia permitindo aumentar o sucesso terapêutico e diminuir a morbilidade e mortalidade associadas ao cancro oral.

Finalmente é importante nunca esquecer a importância do diagnóstico precoce do cancro oral. Atrasos na identificação e reconhecimento de lesões suspeitas são responsáveis pelo atraso do diagnóstico e pela fase avançada da doença no diagnóstico com taxas de sobrevivência muito baixas.

Palavras-chave:

Invasão perineural; Invasão perineural e cancro oral; Carcinoma oral espinho celular.

Methods:

A web-based search for all types of articles published was initiated using MEDLINE/PubMed, with the key words “oral cancer”, “perineural invasion”, “oral squamous cell carcinoma” and “perineural invasion in oral cancer”. The search was subsequently refined. The sites of specialized scientific journals in the areas of oral and maxillofacial surgery, oral medicine, and oncology were also used. Information was then selected concerning perineural invasion, prognostic and clinical meaning in oral cancer.

Introduction

Cancer is one of the most common causes of disability and mortality worldwide, with more than 10 million new cases and more than 6 million deaths each year[7]. According to the World Health Organization (WHO) by 2020 there will be 15 million new cancer cases and 10 million cancer deaths every year[7].

Head and neck cancers is an aggressive epithelial malignancy, that collectively account for approximately 10% of the world’s total new cancer cases with oral

squamous cell carcinoma (OSCC) being the responsible for more than 90% of all oral cancer[1,4,10,56,57,61,62].

Oral cancer (OC) is the usual designation of a subgroup of head and neck malignancies that can develop in various locations of the oropharynx and oral cavity, such as lips, tongue, salivary glands, floor of the mouth and buccal mucosa, according to the International Classification of Diseases (ICD version 9)[66]. Oral cancer holds the eighth position in the cancer incidence ranking worldwide[1,7,8]. However, oro-pharyngeal cancer is more common in developing than developed countries (according to the WHO). Incidence rates for oral cancer vary. For instance in south-central Asia it ranks among the three most common types of cancer. And there has also been a sharp increase in the incidence rates in several countries and regions such as Denmark, France, Germany, Scotland, central and eastern Europe and to a lesser extent in Australia, Japan, New Zealand and the United States of America[7].

Despite the achieved therapeutic advances in surgery, radiotherapy and chemotherapy, the five-year survival rate for oral cancer has not improved significantly over the past decades and it remains at about 50 to 55%[1,4].

Biological behaviour of OSCC is uncertain because there are a great number of tumours that in the initial state show an aggressive biological behaviour with early regional metastasis and death. In contrast, other tumours in advanced state can slowly metastasize after treatment and, patients remain free of disease for a long period of time[2].

Risk and Prognostic Factors

Researchers began to look for factors that could influence prognostic of oral cancer in order to try to prevent it. These factors can be related to patient, such as sex and age, diet, oral health, tobacco and alcohol consumption, socioeconomic conditions and diagnostics delays; related to tumour itself, like anatomic site, disease staging, tumour thickness, extracapsular spread, histologic differentiation, perineural invasion, angiogenesis, molecular markers, oncogenes or human papillomavirus, and related to the treatment, such as cervical node dissection and resection margins[1,2].

Dietary factors have been thought to account for about 30% of cancers in western countries, making diet the second most preventable factor only overcome by tobacco use. The contribution of diet to cancer risk in developing countries has been considered to be lower, perhaps around 20%[7]. Regarding oral health, poor condition of basic mouth hygiene such as poor dentition, regular mouthwash use (with alcohol), lack of

toothbrush use and never having a dental check-up are risk factors for head and neck cancers, independent of tobacco use and alcohol consumption[9].

Regarding tumour itself, data from literature indicates that the presence of lymphovascular and perineural tumour invasion (PNI) as well as increased depth of subepithelial invasion needs to be viewed as important prognostic factors in addition to traditional diagnostic characteristics, such as tumour size, lymph node involvement, and the presence of distant metastases[15], because the mere presence of PNI has important prognostic significance[16,18,20]. PNI in head and neck squamous cell cancer has been associated with poor outcomes and with poor prognostic[19,30.].

According to the results of Rahimaet al (2004) and Milleret al (2012) PNI is associated with tumour thickness, differentiation, and lymph node metastasis and is an important predictor of recurrence and being a poor prognostic and survival factor in patients with SCC of the oral cavity and oropharynx, which is in accordance with other studies[16,18,19,20,42] .

Perineural Invasion

Perineural Invasion is a form of metastasization or tumour spread similar to but distinct from vascular or lymphatic invasion, that is also called “neurotropic carcinomatous spread” and “perineural spread”[10,13,16]. It is as well the process of neoplastic invasion of nerves and an under-recognised pathway of metastatic spread. It can be a source of distant tumour spread well beyond the extent of any local invasion and for some tumours it may be the sole pathway of metastatic spread[10,13]. Although it is important to notice that some authors believe that is unclear whether squamous cell carcinoma shows a tendency toward perineural invasion[18].

Among the various parameters used to predict the outcome of malignant disease, PNI is in wide use as an indicator of aggressive behaviour[10,11,13,15,20]. PNI is generally considered as a risk factor for survival of patients with primary head and neck cancer and is considered an indicator for adjuvant treatment[11,16]. Perineural invasion in oral squamous cell carcinoma is recognized as a significant predictor of outcome[39]. It is associated with aggressive behaviour and greater risk of recurrence and metastasis[42]. PNI is a prognostic indicator for treatment failure and poor outcome in the management of OSCC and indicates the need to determine mechanisms that are involved with it[39].

The outcome of patients with squamous cell carcinoma of skin, lip and oral cavity is adversely affected by the presence of PNI, and PNI of major nerves is associated with locoregional recurrence and decreases survival in patients with squamous cell carcinoma of the upper aerodigestive tract[42]. Because PNI has been associated with

increased rates of nodal recurrence, some authors have suggested the need for elective neck dissection in PNI-positive, clinically node-negative tumours[16]. The presence or absence of PNI was associated with nodal positivity or negativity, respectively according to Miller et al (2012)[16].

Despite the increasing recognition of this metastatic process, there has been little progress in the understanding of molecular mechanism behind PNI and no target treatment modalities aimed at this pathologic entity[13]. Perineural spread of malignancy is not a routine feature of OSCC but it is more commonly associated with tumours like adenoid cystic carcinoma[9].

It is important to highlight that it is emerging as an important pathologic feature of many malignancies like those of the pancreas, colon and rectum, prostate, biliary tract, stomach, head and neck[2,13]. Despite the high incidence of PNI in many tumour types, its true prognostic significance still remains difficult to recognise, due to the objective difficulties in identifying nerve invasion. Thus, PNI's incidence in different tumour types varies considerably, depending on the method of evaluation. Improvement in the recognition of PNI during histological routine analyses may help identifying tumour–nerve interaction as a key pathologic feature, establishing its true prevalence in various malignancies[20].

Regarding PNI's incidence in OSCC data are very different depending on their source. For example Binmadiet et al (2011) refers the existence of a marked variation in the frequency of PNI ranging from 2% and 30% to a high of 82%[10]. Petrie et al (2010) says that head and neck SCC produces PNI in approximately 2.5% to 14%[41]. For Kurtz et al (2005) the reported incidence of finding perineural invasion has ranged from approximately 5% to 50%[19].For Marchesi et al (2010) incidence of 80% of PNI in head and neck cancer[20] and for Rahima et al (2004) the occurrence of PNI is reported to range from 6% to 30%[42]. In the study of Fagan et al (1998) PNI was diagnosed in 52% of all tumours[40] and for Brennand-Roperoccurs et al (2010) PNI occurs with a reported frequency of 24% of patients with cancer of the head and neck[63].

It was also found a correlation between PNI and depth of invasion with an increase in depth tumour cells coming in contact with more nerve fibers and more easily enter into the perineural space and continue to grow along nerves[42].

Some investigators have reported a relationship between PNI and the incidence of cervical lymph node metastasis and also that PNI is one of the independent factors that predict cervical lymph node metastasis[40,42]. This suggests that tumours that invade the perineural space are biologically more aggressive[42]. Therefore, tumours that show PNI

should have elective neck dissection because the rationale of elective neck dissection is based on the hypothesis that metastases may progress sequentially from primary tumours to regional lymph nodes before spreading to distant sites[42]. That is why PNI should be considered as a tumour marker that signifies a different, more aggressive biologic growth and metastatic behaviour, that needs more aggressive resection, coincident management of neck lymph nodes, and the addition of adjuvant therapy[41,42].

Another important aspect concerning PNI by OSCC is that it isn't always manifested as neurologic deficit. Neurologic deficit can occur suggesting PNI but it is not a direct correlation. In terms of diagnosis, perineural involvement of OSCC cannot happen by clinical and radiological means alone. Therefore, histological examination of the specimen should be carried out routinely to confirm neural involvement so as to plan any adjuvant therapy to improve locoregional control, as perineural invasion suggests a bad prognosis[49].

Perineural Invasion: concept evolution

Different theories have been proposed to explain the exact nature of PNI[10]. It was first described by Cruveillerin 1835 in head and neck cancer[10,13,64]. Until the middle of 1980, conclusions were divided as to whether PNI was or was not an unfavourable prognostic indicator[17].

Despite the fact that it has been identified for more than 150 years, the mechanism of PNI is still poorly understood and no treatments have been developed to target this pathologic entity[1]. In 1985, Batsakis gives a board definition: "tumour cell invasion in, around, and through the nerves"[13,16]. According to Liebeg et al (2012) it is the finding of tumour cells within any of the 3 layers (epi, peri or endoneurium) of the nerve sheath[13]. For Marchesi et al (2010) tumour cells invade both the epineurium and perineurium and may reach the endoneurium, becoming intimately associated with Schwann cells and nerve axons[20]. Other authors have proposed another definition: that at least 33% of the circumference of the nerve should be surrounded by tumour cells and less than that 33% represents focal abutment and not invasion[13].

It has been long presumed to be simply the growth of tumour cells along a "path of low resistance"[10,20,40]. But PNI is rather considered the result of an active and specific reciprocal interaction between peripheral nerves and malignant cells present in perinerium space of local peripheral nerves[20].

For many years PNI has been considered as an extension of lymphatic metastasis, due to the presence of lymphatic channels inside the epineurial layer. However, the demonstration that lymphatic vessels do not penetrate the epinerium excludes that PNI

may be the manifestation of lymphatic metastasis[20,40]. At one time presumed to be a lymphatic channel, the perineural space is now considered an artificial space created during tissue processing[40].

It is clear that a better understanding of the molecular and biological mechanisms involved will be necessary if we consider that PNI could be a new therapeutic target for cancer[10]. Although great interest has been shown in developing molecular markers for PNI, results remain preliminary, with markers lacking specificity, sensitivity, and predictive value[16].

It is important to notice that a distinction has been made between PNI and perineural spread, the former being a microscopic feature of malignancy often confined to the main tumour mass, the latter the clinico-radiological observation of distant spread via perineural spaces, or within the neural sheath and nerve itself[17].

A great difficulty arises from the fact that there is no accepted or standardised definition of PNI among all disciplines[10,13,18,19,25].

Perineural Invasion: relation with recurrence

Although the presence of PNI has been shown to affect prognosis, few studies have examined how the extent of PNI affects recurrence[16].

Binmadi et al (2011) and Miller et al (2012) both agree that PNI is correlated with late stage disease[10,16]. It is also accepted that PNI is associated with locoregional recurrence and decreased survival of patients with head and neck squamous cell carcinoma[39]. In oral SCC, recurrence rates after treatment have been reported to fall in the range 25–48%[18]. According to some authors, these high levels of recurrence rates result from the deeply infiltrative nature of these tumours and their potential for occult neck metastasis and an overall decrease in 5-year survival rate[10,18]. This is a suggestion that these tumours are biologically more aggressive and may often reccur locally[40]. Due to his anatomical location OSCC exhibiting PNI into major named nerves correspond to a unique challenge for surgeon[10].

PNI of both large named nerves and small-diameter (<1 mm) nerves is an independent risk factor for locoregional recurrence and decreased survival of patients with head and neck squamous cell carcinoma. The association between PNI and local recurrence is independent of tumour stage, adjuvant chemotherapy and radiotherapy, tumour margins, vascular or lymphatic invasion, nerve diameter, and whether PNI was present within or peripheral to the tumour according to Fagan et al (1998)[40].

Cancer cell migration towards nerves and then along the nerve trunk within the perineural space likely requires activation of numerous signaling pathways involving trophic factors, extracellular matrix adhesion proteins and regulators of chemotaxis[10].

In OSCC, Kolokythas et al (2010) have shown that the expression of nerve growth factor (NGF: a member of the neurotrophin family that is associated with survival and signaling in many neural cell types) and its receptor – (TrkA: receptor tyrosine kinase A), is correlated with the development of PNI[10,39] (Table 1).

Table 1 - Molecular factors exhibiting significant correlation with PNI in OSCC.
(Adapted and modified from BinmadiN, et al., 2011)

Molecular Factor	Basic Role	Notes
Nerve growth factor (NGF)/tyrosine kinase A (TrkA)	Neurotropic factor	Statistically correlation of NGF and TrkA expression in the cytoplasm of malignant OSCC cells in tumors with histologic evidence of PNI
Neural cell adhesion molecule (N-CAM)	Neural cell surface glycoprotein belonging to the immunoglobulin superfamily of adhesion molecules	Positive correlation between the presence of N-CAM and PNI in OSCC
ICAM-5	Adhesion molecule	Overexpression of ICAM-5 plays a role in OSCC tumorigenesis
Claudin-1	Tight junction protein	Claudin 1 overexpression is associated with angiolymphatic and neural invasion, consisten with aggressive tumor behavior
Claudin-4	Tight junction protein	Strong expression of Claudin 4 was associated with decreased PNI

Laminin-5	Component of basement membrane of skin and mucosa	Significant correlation between staining of laminin-5, an important extracellular matrix protein required for efficient cell motility and the presence of PNI in OSCC
Activin A	TGF-β family cytokine	Correlated with positive N stage, poor histological differentiation and PNI
Bim/Bod, BAG-1	Bcl-2 family of apoptosis regulators	Increase expression associated with the presence of PNI
p73	Tumor suppressor elonging to p53 gene family	Associated with distant metastasis and neural and vascular invasion
Snail	Transcription factor important in the epithelial mesenchymal transition during tumor progression	Snail positive tumors were strongly associated with lymphovascular invasion but not PNI

Cancer-induced inflammatory response leads to the expression of many inflammatory mediators including NGF, which could then facilitate tumour dissemination, cancer cell survival in microenvironment of distant organ as well as PNI. This involves cancer cells chemotaxis, proteolytic degradation of protective nerve sheath, and active motility of cells along nerves. PNI is affected by factors that influence migration, inflammation, wound healing, angiogenesis and cancer invasion[39,20].

Nerve-specific adhesion complexes and extracellular matrix proteins have been implicated as molecular determinants of PNI[10] (Figure 1). Adhesion molecules not only mediate cell binding but also activate signal transduction pathways associated with morphogenesis in certain physiologic and pathologic conditions. Alterations in cell–cell and cell–matrix adhesions are considered to be important factors in OSCC growth and dissemination[10]. It seems that, a higher expression of adhesion molecules is expected in well-differentiated OSCC, where these cells maintain their cellular cohesiveness and are less invasive than in poorly differentiated OSCC[46]. However some studies have

been unable to establish an association between the expression of adhesion molecules and metastasis[46]. In addition, proteins that control cell growth, differentiation and apoptosis also have been identified as correlating with PNI[10] .

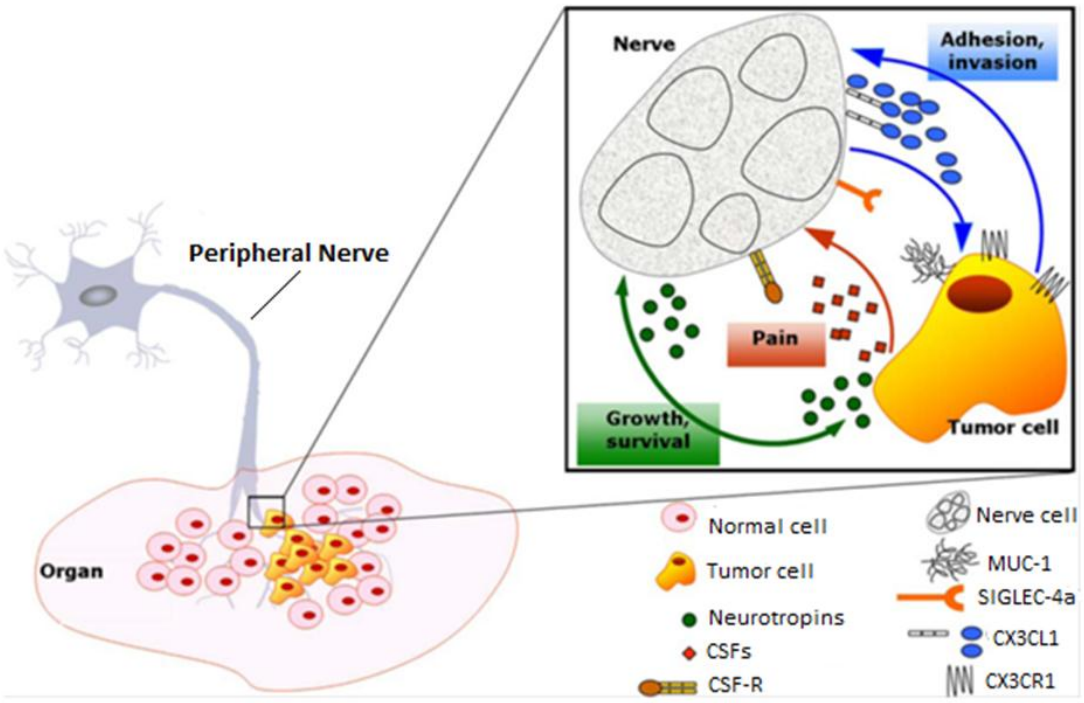

Figure 1 - Schematic representation of some of the molecular mechanisms involved in perineural invasion. A nerve inside a peripheral organ (e.g. pancreas, prostate) developing a tumor is represented. Tumour cells adhere to neural cells, infiltrating the perineural space. Molecules involved in this interaction include the chemokine Fractalkine/Neurotactin (CX3CL1) expressed by neurons and its receptor CX3CR1 on tumor cells and membrane-bound glycoprotein (SIGLEC-4a) binding to tumortumour mucins (MUC-1).
(Adapted and modified from Marchesi F,et al., 2010)

Axogenesis, neurogenesis and cancer

Emerging models of PNI strongly suggest that interactions between tumour cells and nerves not only induce tumour cell migration but also stimulate axonogenesis or enlargement of nerves axon extension, or increased axon number, and neurogenesis an increase in neuron body cell numbers, that can lead to increased nerve density in and around neurotropic malignancies. This process is a newly recognized phenotype for tumour progression which is important in many normal physiologic processes such as growth, development and wound healing[10]. In pancreatic cancer some findings indicate a mutual tropism and paracrine interaction between neurons and cancer cells,

nerves provide a prosperous environment for tumour growth and the interaction gives beneficial effects on growth of both nerves and tumour[20].

It has been reported that the size of nerve fibres adjacent to pancreatic tumour are augmented, indicating a mutual advantage between tumour cells and nerve ending. The increase in neuritis formation suggests that neurotropic factors such as NGF and brain-derived nerve growth factor (BDNF), growth factors and axonal guidance molecules can be key molecules in this crosstalk[20]. Additionally a PCR analysis on mycrodissected surgical sections of prostate cancer has revealed that neurotropins are up-regulated in tumour cells as well as in intratumoural nerves[20].

In head and neck squamous cell carcinoma the expression of laminin-5 by tumour cells was found to significantly correlate with PNI, supporting the concept that the deposition of basement membrane constituents may be required in the process of nerve invasion[20].

As a conclusion it can be said that PNI is not well studied. A lack of experimental models or even an accurate definition for PNI has hindered progress towards understanding the mechanisms of this phenomenon. With a better understanding of the mechanisms involved in it new therapeutic agents or strategies can be developed to target this form of tumour spread[10].

Perineural Invasion in different types of oral cancer

Several types of oral cancer can be described and their relation with perineural invasion differs. Tongue is considered one of the most common locations of oral cancer and exhibits high rates of metastasis[46]. Squamous cell carcinoma of the tongue exhibits some peculiarities and is often more aggressive and characterized by fewer differentiated infiltrating cells. It was also observed that the presence of PNI, but not lymphovascular invasion (LVI) had a significant increase in mortality among those patients with tongue cancer[15].

The survival rate is estimated at 5 years and is inversely proportional to its detection and it was demonstrated that 90% of local and regional neck recurrences for oral floor cancer occur within the first 2 years[56]. The presence of neck node metastasis is the most important predictor survival factor and regional recurrence after surgical removal is the most frequent cause of treatment failure[46].

Miller et al (2012) made the first study that went beyond the dichotomous variable of PNI positivity or negativity and to demonstrate that the extent of PNI is correlated with patient outcome. According to these authors, perineural invasion can be subcategorized as IT (intratumoural), peripheral or ET (extratumoural)[16]. Also,

according to their findings, the presence or absence of PNI was not significantly related to the length of disease-free survival. However the maximum extent of PNI was significantly correlated with the length of time period of disease free survival[16] .

Lip cancer, which is the second most frequent oral cancer, with squamous cell carcinoma (SCC) type being the most frequent (95% of all cases)[61]. Histological evidence of neural involvement has been demonstrated in 2% of cases of SCC of the lower lip and is a poor prognostic indicator. Such tumours can spread axially in a centripetal and centrifugal manner with extension along cranial nerves and subsequent invasion of the central nervous system[63].

Another cancer of head and neck cancers is cutaneous squamous cell carcinoma of the head and neck (SCCHCN). PNI occurs in SCCHCN spreading into the brain stem. When the tumour invades the perineural space of a cranial nerve, it can extend locally or distally in the nerve sheath and eventually reach the brain stem[12]. PNI in SCCHN is associated with decrease survival[12]. Panizza et al (2011) supports the notion that, in this tumour, appropriately planned surgery may improve survival. Also, a surgical resection with negative margins offers a patient with PNI a better chance for cure. In this kind of cancer it is made a separation between clinical PNI or histological PNI. Clinical PNI occurs when the perineural space of a named branch of a cranial nerve is invaded and this invasion can be visualized in a MRI. These patients present with neurologic deficits related to the affected nerve. Histological or incident PNI, on the other hand, occurs when nerve invasion is found incidentally on a surgical specimen. This distinction is paramount because it has significant therapeutic decisions. The authors also concluded that MRI demonstrated the presence and anatomic extent of perineural spread in most cases. In conclusion, clinical PNI must be accompanied by MRI findings[12].

Regarding oral salivary gland cancers, they account for 2-3% of all malignant neoplasms of the upper aerodigestive tract and up to 20% of all salivary gland tumours[33], yet they are the most diverse with at least 24 different types recognized by the World Health Organization (WHO)[23]. This diversity combined with the rarity of many of the tumour types and the unpredictability in long-term outcome imposes a significant challenge on the management of salivary gland malignancies overall[23]. Mucoepidermoid cancer (MEC) represented the most common oral cancer of salivary gland type in previous series (50%), followed by adenoid cystic carcinoma (ACC, 25%) and polymorphous low-grade adenocarcinoma (PLGA, 20%), where as acinic cell carcinomas, adenocarcinomas, not otherwise specified and myoepithelial cancers were rare. According to Schwartz et al (2011) ACC and PLGA show similar frequency of

perineural invasion, limited regional lymph node metastasis and locoregional disease relapse. However, compared to PLGA, ACC tends to present a higher stage disease being associated with residual local disease and give rise to rare distant metastasis[33].

From a differential diagnostic point of view, PLGA have to be, if possible and clinically relevant, differentiate from ACC and, most importantly, from pleomorphic adenoma. Pleomorphic adenoma lacks evidence of peripheral invasion into surrounding tissue and perineural invasion[33]. Thus, it is of great importance to carefully examine the tumour periphery in excision biopsies looking for evidence of infiltration into surrounding salivary gland and other normal tissue[33]. Malignant tumours of oral minor salivary gland origin are rare, but constitute an important area in the field of oral pathology as they are mostly considered to behave clinically better than squamous cell carcinoma, the most frequent malignant tumour of the oral cavity[33].

Adenoid Cystic Carcinoma (ACC) is a rare tumour entity and comprises about 1% of all malignant tumours of the oral and maxillofacial region[24,31]. Early diagnosis is important because these are slowly growing tumours that produce diffuse invasion and with the potential to produce distant metastases, mainly to the lungs and bones[24,28,30,31].

Salivary gland ACC has indolent behaviour, but is prone to late local recurrence and presents a strong neurotropism, with a tendency to invade nerves adjacent to the lesion with a predilection for perineural spread[17,18,27,31].

More recent gene expression profile of ACC has focused on tumour stage, histological grade, metastatic progression, early development, apoptosis and cell cycle regulation. However, gene expression profile of ACC associated with PNI is still sparse, as a prognostic marker[30]. Initiation and progression of PNI in ACC is multifactorial and a multistep process accompanied by accumulation of alterations. Delineating these genes involved may lead to important new insights into carcinogenesis of ACC[30].

LCM (laser capture microdissection) affords the opportunity to perform molecular genetic analysis of pure populations of ACC cells in their native tissue environment[30]. It was demonstrated that, using carefully controlled conditions, in vivo subpopulations of malignant cells from ACC with PNI can be screened for tens of gens. These genes can have potential roles in the pathobiology of ACC associated with PNI. Future work focusing on the function of these genes in normal and malignant salivary gland tissues or therapies based on functional gene ontology relationship will hopefully lead to improved outcomes for patients with ACC[30]. Chen et al (2007) report the first application of combining LCM and cDNA microarray technologies to analyse gene expression in clinical cancer specimens and to correlate the profile with PNI[30].

Intracranial ACC is even more rare and has been reported as 4 – 22% of ACC. It could be primary or secondary which could occur either by direct invasion, hematogenous spread, or perineural spread[24].

Unlike most cancers, most patients with ACC survive for 5 years, only to have tumours recur and progress. In a recent study of a cohort of 160 ACC patients, disease-specific survival was 89% at 5 years but only 40% at 15 years. Therefore, these patients require long-term follow-up[31].Regarding the signs and symptomatology of this entity, they are related to the anatomical site of the lesion and include facial pain, parasthesia in trigeminal distribution is commonly reported reflecting the frequency of involvement of gasserian ganglia, and possibility of perineural spread along the trigeminal nerve[24].Literature is consistent that the time between onset of neurological signs and symptoms, and the time of diagnosis range between few months to 3 years[24].

Salivary Adenoid Cystic Carcinoma and Perineural Invasion (known mechanisms so far)

Though the mechanism of its invasion and metastasis still remains undefined to some extent, evidence suggests that progression of Salivary Adenoid Cystic Carcinoma (SACC) is affected by varied expression of some growth factors and their receptors, which also impact the prognosis of patients with SACC[32]. It has been reported that there is an overexpression of transforming growth factor-alpha (TGF-α) and epidermal growth factor receptor (EGFR) in adenoid cystic carcinoma (ACC), and that the level of EGFR is higher in the pathological subtype of greater malignancy and associated with worse prognosis. NGF modulate growth, development and regeneration of many types of nerve cells in the central and peripheral nervous system, but it can also influence proliferation and differentiation of tumours, including head and neck neoplasms. Recently, a few experiments have shown that NGF or its receptor is highly expressed in some malignancies with neurotropism such as prostatic cancer and also specifically elevated in oral squamous cell carcinoma with perineural invasion. In addition, NGF overexpression is associated with lymph node metastasis, distant metastasis, higher TNM stage and lowered survival of patients with squamous cell cancer of head and neck[32].

Vascular endothelial growth factor (VEGF) is the most potent mitogen of vascular endothelium. VEGF is able to promote differentiation, proliferation and migration of endothelial cells, stimulates neovascularization, and can induce invasion and metastasis of tumour cells. Among patients with malignancies, including head and neck squamous cell carcinoma, high VEGF expressers have a worse prognosis than low

expressers. Though increased expression of NGF and VEGF has been demonstrated in the growth, development and regeneration of tumours often associated with perineural or perivascular invasion, and though these two factors have been suspected to play critical roles in the progression of SACC as well as in assessment of the prognosis, it has not been clarified how the two factors influence the invasion and metastasis of SACC. There are also some reports of their direct contribution to the prognosis of patients[32]. Some have found that PNI signify a worse prognosis yet others find that perineural involvement does not impact the outcome[32].

Results of the study by Li et al (2010) showed that the immunoreactivity of NGF was relatively strong in SACC cells, with the positive rate higher in the solid than in the cribriform-tubular type, suggesting that SACC cells expressed NGF and the staining intensity correlated with pathological subtypes of different malignancy. Meanwhile, they also observed that there was obvious heterogeneity in NGF expression between the PNI and the non-PNI groups, suggesting that it was associated with neurotropism. The probable explanation is that NGF expressed by ACC cells is attracted by NGF receptors in nerve tissues, causing tumour cells and nerves to grow together. In addition NGF expression induces more neovascularisation around neurofibres, which supplies nutrients for tumour growth, promoting faster proliferation of the tumour cells around and perineural invasion. In this study, the NGF positive rate was higher in the advanced-stage and the recurrence groups than in the early-stage and the recurrence-free groups, suggesting that in the procession of SACC, more NGF could be expressed, and invasiveness of tumour cells enhanced. Other than the explanation above, another proposed mechanism is that NGF promotes invasion and metastasis of neoplasms through mediating depho-sphorylation of serine in actin-binding proteins leading to tumour cell movement, as well as stimulating tumour cells to produce more heparinase degrading heparan sulfate proteoglycan which is substantial in extracellular matrix. Those analyses demonstrated that the prognosis of non-PNI, recurrence-free, cribriform-tubular and early-stage patients was better in those whose specimens NGF level was lower. In multivariate analysis indicating that the prognosis of patients with high NGF expression differed significantly from that of those who lacked it as NGF expression was elevated, the mortality risk of SACCs increased[32]. In the same study, it is showed that staining of VEGF was more intense in the perineural invasion than in the non-perineural invasion group, the distribution of which was similar to that of NGF. Still, the correlation between these two factors in SACC tissues requires further studies[32].

In conclusion, staining intensity of NGF and VEGF was stronger in the specimens of PNI, recurrence, high-malignancy pathological type and advanced TNM stage. Additionally, expressions of these two factors, as well as some pathoclinical factors like PNI, were independent prognostic factors[32].

The results suggest that NGF and VEGF significantly correlate with SACC invasion and metastasis, and they may serve not only as prognostic factors, but also as novel therapeutic targets in SACC[32].

Inhibition of glandular tumor growth - Cimetidine

Cimetidine is the first histamine type-2 receptor (H2R) antagonist to be used clinically. It is commonly prescribed to treat gastro-esophageal reflux disease as well as gastric and duodenal ulcers. It has been reported that cimetidine improves the survival of patients with malignant tumours and inhibit the growth of glandular tumours, however the mechanism of action underlying this effect is unknown[34].

Human salivary gland tumour (HSG) cells spontaneously express neural cell adhesion molecule (NCAM), HSG cell proliferation may be controlled via NCAM-NCAM binding mechanism and NCAM may be associated with perineural invasion by malignant salivary gland tumours. Cimetidine inhibited NCAM expression and induced apoptosis in HSG cells[34].

Cimetidine has been shown to inhibit growth of gastrointestinal cancers via several mechanisms including enhancement of immune activity and inhibition of cancer cell proliferation. Therefore cimetidine may act by enhancing the host immune response against tumour cells or by blocking the cell growth-promoting activity of histamine. But the exact mechanisms by which cimetidine suppresses the development of salivary gland tumors remain to be elucidated[34].

Fuduka et al (2001) demonstrate that NCAM is spontaneously expressed in the human salivary gland tumour. HSG cell line, derived from the submandibular salivary gland, and HSG cell proliferation may be controlled via a NCAM-NCAM binding mechanism. Later, in 2005, they demonstrate that NCAM may be involved in perineural/neural invasion by malignant salivary gland tumours[34].

The same authors (Fukuda et al 2008) found that cimetidine blocks not only salivary gland tumour cell adhesion to neural cells, but also tumour growth by inhibiting the NF-κB-mediated induction of NCAM. They also demonstrate that cimetidine can induce apoptosis in the inoculated tumuor mass in a nude mouse model in which the salivary gland tumour cell line HSG was injected subcutaneously[34].

Neural Cell Adhesion Molecule (N-CAM)

Neural cell adhesion molecule (N-CAM) is a member of the immunoglobulin-like (Ig) superfamily of adhesion molecules. N-CAM, is expressed on the surface of astrocytes and neurons. During development N-CAM affects cell migration and neurite outgrowth. In the adult, N-CAM is expressed at synaptic junctions and is thought to modulate synaptic function and its promoter appears to be activated in response to neural stimulation[35]. In response to neural injury, N-CAM can inhibit the proliferative response by astrocytes and promote axonal regeneration[35,47].

NCAM is a key mediator of structural plasticity in the central nervous system, but the mechanisms that control its expression are unknown[36].

The localization of N-CAM at the synaptic cleft makes it an attractive candidate for signaling alterations in the structure and function of the synapse. Activation of NF-kB can occur in neurons in response to neural stimulation to treatment with glutamate, or to treatment with TNF-a[35].

The identification of a specific factor for predicting clinical outcome in the case of salivary gland carcinoma would be helpful for selecting more effective treatments. The expression of NF-kB and iNOS were all correlated to the PNI[37].

Perineural Invasion: relevance in treatment

Traditionally, radiotherapy is considered postoperative adjuvant for oral cancer in certain situations when the risk of disease recurrence is high. Some factors can be associated with local failure like close or positive surgical margins, extent of lymph node involvement in neck, extracapsular spread, vascular or perineural invasion having the most accurate data with which to determine prognosis or on which to base

treatment decisions is important[1,10,50]. The combination of surgery and radiotherapy is commonly used in the management of oral cancer like squamous cell carcinoma and it is effective in improving locoregional control. Recent data show that, even with these treatments, there are subgroups of patients who still have unacceptable recurrence rates (especially those with positive margins, multiple risk factors and advanced neck disease). There is evidence that concomitant chemotherapy can improve locoregional control and disease-free and overall survival[50].

Usually the surgical procedure employed in the presence of obvious cervical node metastasis has been radical neck dissection. Yet, this procedure is a source of significant postoperative morbidity. Selective neck dissection is highly dependent on the primitive tumour location and according to some authors, apparently achieves similar regional control and survival rates as those attained with more extensive neck dissections[1]. It is also relevant that a strong correlation has been demonstrated between a resection margin free of disease and higher survival rates, with longer time until recurrence of disease[1,2].

It is very important not to forget that the parotid glands are immediately adjacent to the treatment targets for oral cavity cancer, conventional radiation may lead to permanent xerostomia, subsequent oral cavity complications, and poor quality of life[4,62].

Particular disease characteristics such as perineural invasion may alter treatment approach and careful review by specialist pathologists may give guidance as to surgical approach[13,63].

Conclusions

Treatment failures in patients with oral cancer primarily happen because of loco-regional recurrence and distant metastasis. Perineural invasion (PNI) in tumours occurs in several human malignancies and has been considered as an independent prognostic factor. Analyses of a number of tumour types have shown that PNI is a histological marker for aggressive disease, which identifies patients with worse clinical outcome and may add important additional information to classical prognostic parameters. Because of the impact of PNI on prognostic and treatment planning, pathologists should routinely comment on the presence of PNI.

An upcoming all-inclusive molecular and clinical staging system will allow a more accurate selection of patients that should undergo more aggressive, specific or individualised cancer therapy. More knowledge and subsequent application of new methods of diagnosis will increase prognostic and therapeutic success, decreasing disability and mortality rates associated with oral cancer.

Still PNI is not enough studied, not even an accurate definition is established which makes the understanding of this entity very challenging. Also several variations in literature were detected regarding the prognostic significance of PNI. Further studies are recommended that will allow us a better knowledge of mechanisms involved in PNI and therefore, new therapeutic agents can be created in order to target this form of tumour spread.

At last it is important never to forget that it is urgent to improve oral cancer early detection by clinicians. Delays in identification and recognition of suspicious lesions contribute to advanced stage at diagnosis and lower survival statics.

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