# Predictors of cervical lymph node metastasis in salivary gland cancer

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**ABSTRACT:** *Background.* This study compares clinicopathological parameters with novel molecular markers for predicting cervical lymph node metastasis in salivary gland cancer.

*Methods.* Three hundred sixteen salivary gland carcinomas were included in this study. Genomic epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), phosphatase and tensin homolog (PTEN), and hepatocyte growth factor receptor (MET) was determined by fluorescence in situ hybridization (FISH). Chi-square tests, multivariate regression, and Kaplan–Meier survival analysis were used for statistics.

*Results.* Nodal staging determines long-term survival. Clinicopathological parameters associated with positive neck nodes are advanced age ($p = .006$), T3/T4 classification, histological high-grade malignancy, and diagnosis of salivary duct carcinoma ($p < .001$ each). Neck node metastases also correlate with copy number gain of EGFR ($p = .004$) and HER2, aberration of MET, and deletion of PTEN ($p < .001$ each). Multivariate analysis showed SDC ($p = .002$) to be the strongest predictor of lymph node metastasis, followed by MET aberration ($p = .009$), T3/T4 classification ($p = .017$), PTEN deletion ($p = .042$), and adenocarcinoma not otherwise specified (NOS; $p = .047$).

*Conclusion.* The histological subtype is crucial for decisions regarding neck dissection. New molecular parameters may also indicate elective treatment of the neck. © 2013 Wiley Periodicals, Inc. *Head Neck* **36:** 517–523, 2014

**KEY WORDS: salivary gland cancer, nodal metastasis, predictors, PTEN, MET**

## INTRODUCTION

Salivary gland carcinomas are rare malignomas that constitute about 5% of all head and neck carcinomas.[1] Because of their enormous histomorphological diversity and variable clinical courses, these tumors represent a major challenge for both pathologists and clinicians in diagnostics and treatment. Metastases of the regional neck nodes are a decisive negative predictor of survival in head and neck squamous cell carcinomas (SCCs) but also in salivary gland cancer.[2–4] Surgical neck dissection is routinely conducted in patients with clinical suspicion of metastasis (therapeutic neck dissection). However, the indication for neck treatment remains unclear if presurgical imaging does not indicate nodal metastasis (elective neck dissection). Currently, increased tumor size and high-grade histology are the main criteria for elective neck dissection.[3,5–7] Recently, we could show the occurrence of genomic aberrations of the tyrosine kinase receptors epidermal growth factor receptor 1 (EGFR), human epidermal growth factor receptor 2 (HER2), and hepatocyte growth factor receptor (MET) as well as of the tumor suppressor gene phosphatase and tensin homolog (PTEN; located on chromosome 10) in different subtypes of salivary gland cancer, which have a strong impact on overall survival.[8–10] The present study compares the predictive value of known clinicopathological parameters with the above-mentioned novel molecular markers for lymph node metastasis in salivary gland carcinomas.

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## MATERIALS AND METHODS

### Patients and therapy

This retrospective study comprised 316 patients (148 men and 168 women; mean age 61.0 years) with a carcinoma of the major or minor salivary gland. All patients had been treated either at the University Hospitals Regensburg or Erlangen/Nuremberg or at the Nuremberg Hospital between 1984 and 2008. The study was a cooperation among the Departments of Oral and Maxillofacial Surgery, Otorhinolaryngology, and Pathology. Clinical data were obtained from charts and tumor registries. All research activities were covered by the votes of the ethics committees of the medical faculties at the Universities of Regensburg and Erlangen/Nuremberg.

Two hundred twenty-four tumors (70.9%) were localized in the parotid gland, 46 tumors (14.6%) in the

TABLE 1. Most frequent histologies and lymph node metastasis.

| Histology | Lymph nodes, no. of patients (%) | | | Total |
|---|---|---|---|---|
| | N0 | N1 | N2–3 | |
| Total | 217 (68.7) | 37 (11.7) | 62 (19.6)* | 316 |
| ACN | 40 (93.0) | 3 (7.0) | 0 | 43 |
| ACC | 41 (82.0) | 4 (8.0) | 5 (10.0) | 50 |
| MEC | 38 (74.5) | 7 (13.7) | 6 (11.8) | 51 |
| SDC | 10 (28.6) | 6 (17.1) | 19 (54.3) | 35 |
| Adenocarcinoma NOS | 20 (55.6) | 2 (5.6) | 14 (38.9) | 36 |
| SCC | 17 (56.7) | 7 (23.3) | 6 (20.0) | 30 |
| Myoepithelial carcinoma | 15 (71.4) | 1 (4.8) | 5 (23.8) | 21 |
| Polymorphous low grade adenocarcinoma | 9 (69.2) | 4 (30.8) | 0 | 13 |
| Others | 27 (73.0) | 3 (8.1) | 7 (18.9) | 37 |

Abbreviations: ACN, acinic cell carcinoma; ACC, adenoid cystic carcinoma; MEC, mucoepidermoid carcinoma; SDC, salivary duct carcinoma; NOS, not otherwise specified; SCC, squamous cell carcinoma.
* Including 2 N3 cases.

submandibular gland, 44 tumors (13.9%) in the minor glands, and 2 tumors (0.6%) originated from the sublingual glands. Primary curative resection (conservative or radical) was conducted in 293 patients (92.7%); 156 total parotidectomies (53.2%), 35 partial parotidectomies (11.9%), 46 submandibulectomies (15.7%), and 56 local tumor resections (19.1%). Salvage tumor resections were conducted in 8 patients (2.7%). Fifteen patients (4.7%) received primary radiotherapy, radiochemotherapy, or single chemotherapy. After therapy, 245 patients showed clear or close resection margins (R0), 48 patients had microscopic residual tumors (R1) and 23 patients had macroscopic residual tumors (including distant metastases, R2). Neck dissection was carried out in 234 patients (74.1%), in 61 patients as radical neck dissection and in 173 patients as selective or modified radical neck dissection. In selective neck dissections, Robbins levels II to IV were regularly removed for parotid tumors, levels I to III for submandibular and sublingual tumors, and levels I to IV for minor gland tumors. Fifty-one patients (16.1%) did not receive any neck treatment because of small tumor size (T1 or T2) and clinical N0 status. In 31 patients (9.8%), neck dissection was not conducted for palliative or other reasons. Postoperative radiotherapy or radiochemotherapy was applied in 185 patients (58.5%) with high-grade histology, positive resection margins, lymph node metastasis, or distant metastasis.

Disease in all patients was retrospectively staged according to the pathological TNM classification of the American Joint Committee on Cancer 2010.[11] If no neck dissection was carried out in patients with a low-size and low-grade carcinoma without any suspicious neck nodes shown in the preoperative imaging, we used the clinical N classification (cN0). The mean follow-up period of all patients was 4.95 years (range, 0.1–21.2 years). Local tumor recurrence or distant metastasis after therapy with curative intention was recorded in 26.6% of patients (78 of 293). Primary or secondary distant metastases (mainly lung) were observed in 29 patients (9.2%). Seventy-eight patients (24.7%) died because of disease-related reasons. The 5-year and 10-year disease-specific survival rates of all patients were 74.0% and 68.5%, respectively.

## Histology

Hematoxylin-eosin stained slides from paraffin wax-embedded tumors were available for all patients. The slides were independently reviewed by 2 experienced pathologists (S.S.-F. and A.A.) without any knowledge of the initial diagnosis. All tumors were classified according to the contemporary World Health Organisation's classification of salivary gland tumors.[12] The study cohort comprised 43 acinic cell carcinomas (ACNs), 50 adenoid cystic carcinomas (ACCs), 51 mucoepidermoid carcinomas (MECs), 35 conventional (high-grade) salivary duct carcinomas (SDCs), 36 high-grade adenocarcinomas not otherwise specified (NOS), 30 SCCs, 21 myoepithelial carcinomas, 13 polymorphous low grade adenocarcinomas, 9 basal cell adenocarcinomas, 10 oncocytic carcinomas, 5 epithelial-myoepithelial carcinomas, 5 primary malignant mixed tumors, 4 undifferentiated carcinomas, 2 large cell carcinomas, and 2 cystadenocarcinomas. The less frequent tumor entities basal cell adenocarcinoma, oncocytic carcinoma, epithelial-myoepithelial carcinoma, malignant mixed tumor, undifferentiated carcinoma, large cell carcinoma, and cystadenocarcinoma are grouped together under the rubric "others'' in Table 1. All cases of SCC were classified as of primary salivary gland origin after intensive staging procedures (CT or MRI of the head and neck, panendoscopy, X-ray or CT of the chest, and ultrasonography of the abdomen) and exclusion of metastasis to the salivary gland from primary SCC at other sites. Squamoid variants of MECs were thoroughly discarded.[13] Grading was based on a 3-tiered grading system.[4,10,14] ACN, basal cell adenocarcinoma, epithelial-myoepithelial carcinoma, cystadenocarcinoma, and polymorphous low grade adenocarcinoma were considered low grade (G1) with the exception of dedifferentiated tumors, which – together with SDC, SCC, malignant mixed tumor, oncocytic carcinoma, undifferentiated carcinoma and large cell carcinoma – were classified high-grade (G3). MECs were graded according to the criteria proposed in the current World Health Organisation classification.[15] ACCs were divided into predominantly tubulocribriform (G2) and predominantly solid (G3) tumors. Grading of adenocarcinoma NOS and myoepithelial carcinoma was based on nuclear pleomorphism and mitotic activity similar to the Elston and Ellis grading of breast cancer.[16] Thirty-four carcinomas ex pleomorphic adenoma were classified and graded according to the malignant component of the tumor.

## Fluorescence in situ hybridization

As described in detail elsewhere,[17] tissue microarray sections were mounted on charged slides (SuperFrost Plus; Menzel GmbH, Braunschweig, Germany). Hematoxylin-eosin stained tissue microarray sections were used for reference histology. Fluorescence in situ hybridization (FISH) analysis was done by means of directly labeled ZytoLight SPEC phosphatase and tensin homolog (PTEN)/CEN10, SPEC EGFR/CEN7, SPEC HER2/CEN17, and SPEC MET/CEN7 dual color probes (ZytoVision, Bremerhaven, Germany). After probe hybridization, nuclei were counterstained with anti-fading 4′,6-diamidino-2-phenylindole (DAPI) Vectashield (Vector

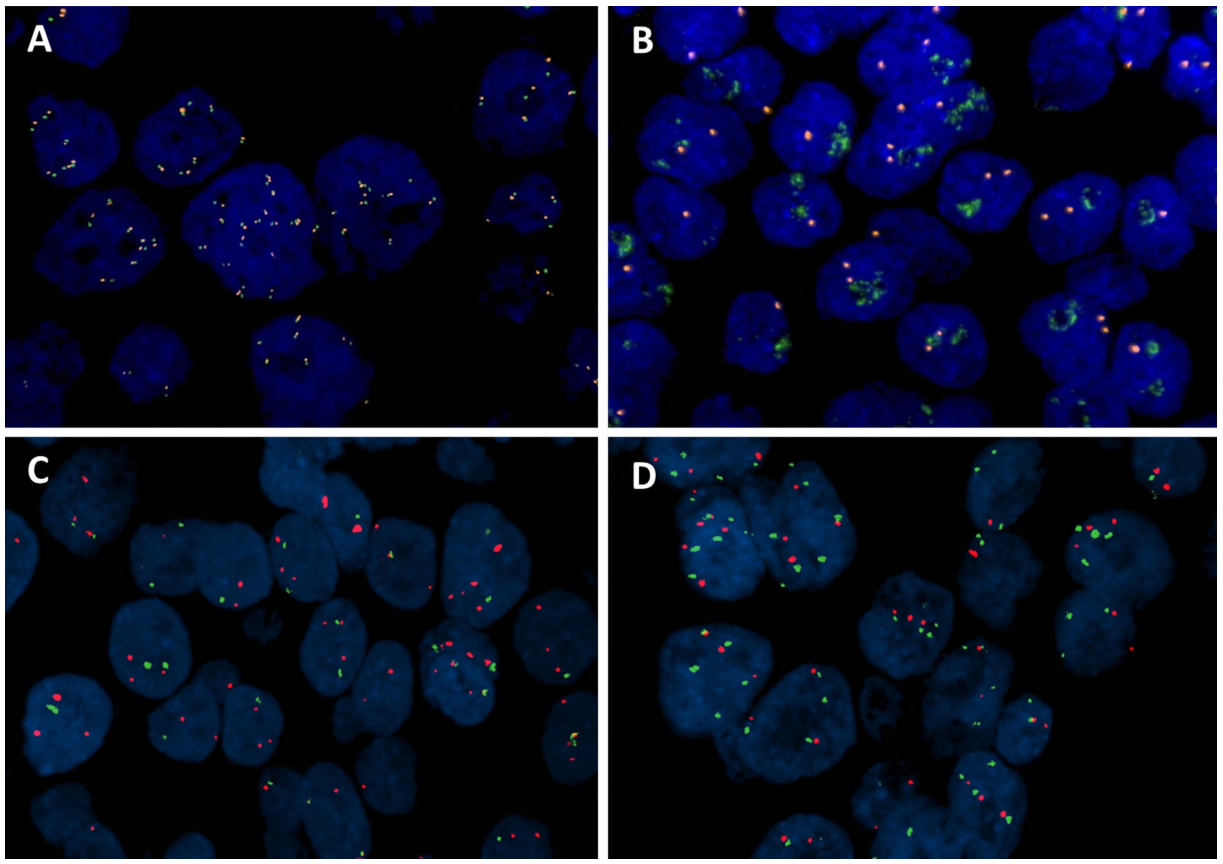


FIGURE 1. Representative examples of fluorescence in situ hybridization (FISH) analysis. (A) High polysomy of epidermal growth factor receptor (EGFR) in adenocarcinoma not otherwise specified (NOS). (B) Cluster amplification of human epidermal growth factor receptor 2 (HER2) in salivary duct carcinoma. (C) Relative hemizygous deletion of phosphatase and tensin homolog (PTEN) in salivary duct carcinoma. (D) High polysomy of hepatocyte growth factor receptor (MET) in mucoepidermoid carcinoma. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Laboratories, Burlingame, CA) and analyzed by epifluorescence microscopy using the AxioImager-Z1 (Zeiss, Göttingen, Germany). Hybridization signals of 50 nonoverlapped nuclei were manually counted on a single cell basis by 2 independent observers. Non-neoplastic salivary gland specimens were used as controls. Representative samples of the FISH analyses are shown in Figure 1 (centromere in red, gene in green).

EGFR and HER2 were evaluated as described before.[8] Samples were grouped as normal disomy, $\leq$2 centromere signals in $\geq$50% of cells; low polysomy/trisomy, $\geq$3 centromere signals in $\geq$40% of cells, excluding cases with high polysomy or gene amplification; high polysomy, $\geq$4 centromere signals in $\geq$40% of cells, excluding cases with gene amplification; and gene amplification, ratio of gene/chromosome $\geq$2 or clusters of probes (>10 copies/tumor cell) in $\geq$40% of cells.[18] Disomy and trisomy/low polysomy were grouped as FISH-negative, whereas high polysomy and amplification (Figure 1) were classified as FISH-positive or copy number gain of EGFR and HER2 in dichotomization.[19,20]

Homozygous deletion of PTEN was defined by the simultaneous lack of both PTEN locus signals and by the presence of centromere signals in >20% of nuclei. We defined hemizygous deletion of PTEN as more than 30% of tumor nuclei containing either 1 PTEN locus signal and $\geq$2 centromere signals or 2 PTEN locus signals and $\geq$4 centromere signals (relative deletions).[10,21]

MET was evaluated in the same way as EGFR and HER2. Additionally, we defined deletion of MET as a ratio of gene/centromere <0.5 in more than 30% of nuclei. Low polysomy, high polysomy, amplification, and deletion were determined as aberration of MET.

### Statistics

Data were analyzed with SPSS for Windows, version 20.0 (SPSS, IBM, Ehningen, Germany). Relationships between parameters were examined using the Pearson's chi-square test ($p < .05$) and the Fisher's exact probability test ($p < .05$) for dichotomized variables. Univariate survival curves were calculated with the Kaplan–Meier method, and we compared distributions by means of the log-rank test. Disease-specific survival was calculated from the date of diagnosis until disease-caused death or end of follow-up. Multivariate logistic regression analysis was conducted (enter method) to determine the effect of predictor variables on regional lymph node disease.

## RESULTS

One hundred twenty-eight patients (40.5%) presented with increased tumor size (T3 or T4). Overall, 54.7% of

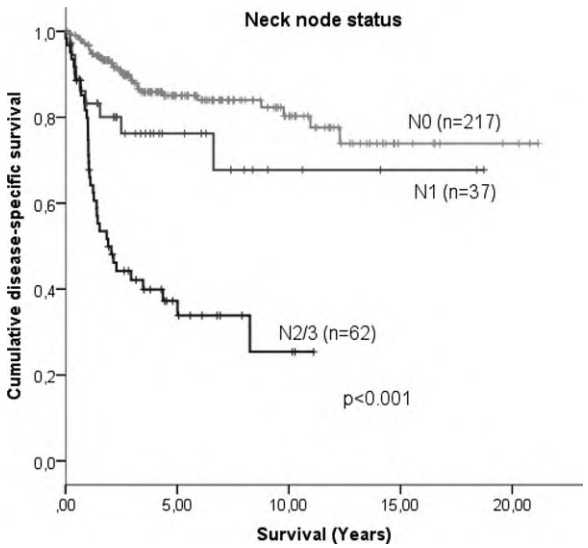


FIGURE 2. Kaplan–Meier survival analysis of the patients' neck node status. N3 status ($n = 2$) is included in N2 status.

the patients had advanced (American Joint Committee on Cancer III and IV) tumor stages. One hundred four carcinomas (32.9%) were classified as low-grade, 50 (15.8%) as intermediate-grade, and 162 (51.3%) as high-grade malignancies. Positive neck nodes were recorded in 31.3% (99 of 316) of the patients, in detail, 11.7% N1, 19.0% N2, and 0.6% N3 (Table 1). In 96 patients, lymph node metastasis was pathologically confirmed. In 3 palliative patients, lymph node metastasis (all cN2) was diagnosed by imaging procedures without any pathological confirmation. Patients with positive neck nodes had a significantly shorter overall survival rate, particularly patients with N2 to N3 status compared to those with N1 status (Figure 2). Disease-specific survival rates for N0, N1, and N2 to N3 status were 85.0%, 76.2%, and 34.0%, respectively ($p < .001$). With view to the most frequent histological subtypes, neck node metastases were rare in case of ACN (7.0%) and ACC (18.0%) but often occurred in connection with SDC (71.4%), adenocarcinoma NOS (44.4%), and SCC (43.3%). SDC and adenocarcinoma NOS very often presented more than 1 positive lymph node. Seven of 15 high-grade MECs (46.7%) presented neck node metastases compared to 16.7% (6 of 36) of low-grade and intermediate-grade MECs ($p = .037$). In ACC, grading (solid vs tubular/cribriform) did not influence lymphatic spread ($p > .05$). Interestingly, 4 of 13 (30.8%) low-grade polymorphous low grade adenocarcinomas showed 1 cervical lymph node metastasis each. Of the investigated clinicopathological parameters, the occurrence of neck nodes was correlated to advanced patient age ($p = .006$), increased tumor size ($p < .001$) and high-grade malignancy ($p < .001$). Forty-four percent (56 of 128) of T3/T4 tumors and 46.9% (76/162) of high-grade variants presented positive neck nodes in comparison to 22.9% and 14.9% for T1/T2 and low/intermediate-grade tumors, respectively. Sex and localization did not show any significant association with lymph node metastasis.

Copy number gain of EGFR (39 high polysomies, 3 amplifications) and HER2 (23 high polysomies, 19 amplifications) was found in 16.9% (42 of 249) and 16.3% (52 of 258) of the carcinomas, respectively. Both positive genomic EGFR ($p = .004$) and HER2 ($p < .001$) were clearly associated with lymph node metastasis in the univariate analysis (Table 2). Hemizygous ($n = 35$) or homozygous ($n = 17$) deletion of PTEN was documented in 23.1% (52 of 225) and strongly correlated ($p < .001$) with positive neck nodes, particularly with N2 status. Aberration of MET (40 low polysomies, 27 high polysomies, 2 amplifications, and 18 deletions) was observed in 38.5% of the patients (87 of 226). A total of 52.9% of the tumors with a MET aberration presented positive neck nodes in comparison to only 19.4% node positivity in tumors with regular genomic MET status ($p > .001$).

The results of the logistic multivariate regression analysis are shown in Table 3. The histological subtype of SDC (odds ratio, 12.32) emerged as the strongest independent predictor of positive nodal disease. Further significant predictors of neck node metastasis were histology of adenocarcinoma NOS, higher T classification, deletion of PTEN, and aberration of MET. In contrast, age, histological grade, EGFR, and HER2 did not show any

TABLE 2. Relation of clinicopathological and molecular parameters with lymph node metastasis.

| | Lymph nodes (no. of patients, %) | | | |
|---|---|---|---|---|
| Parameter | N0 | N1 | N2–3 | Total |
| Total | 217 (68.7) | 37 (11.7) | 62 (19.6)† | 316 |
| Sex | | | | |
| Male | 95 (64.2) | 19 (12.8) | 34 (23.0) | 148 |
| Female | 122 (72.6) | 18 (10.7) | 28 (16.7) | 168 |
| Age | | | | |
| <70a | 147 (75) | 20 (10.2) | 29 (14.8) | 196 |
| >70a | 70 (58.3) | 17 (14.2) | 33 (27.5)** | 120 |
| Site | | | | |
| Gl. parotis | 152 (67.9) | 24 (10.7) | 48 (21.4) | 224 |
| Gl. submand | 30 (65.2) | 8 (17.4) | 8 (17.4) | 46 |
| Minor glands‡ | 35 (76.1%) | 5 (10.9) | 6 (13.0) | 46 |
| Grade | | | | |
| Low/intermediate | 131 (85.1) | 13 (8.4) | 10 (6.5) | 154 |
| High | 86 (53.1) | 24 (14.8) | 52 (32.1)*** | 162 |
| T classification | | | | |
| T1–T2 | 145 (77.1) | 21 (11.2) | 22 (11.7) | 188 |
| T3–T4 | 72 (56.2) | 16 (12.5) | 40 (31.2)*** | 128 |
| EGFR | | | | |
| No copy number gain | 156 (75.4) | 18 (18.7) | 33 (15.9) | 207 |
| Copy number gain | 21 (50.0) | 8 (19.0) | 13 (31.0)** | 42 |
| HER2 | | | | |
| No copy number gain | 163 (75.5 | 22 (10.2) | 31 (14.4) | 216 |
| Copy number gain | 19 (45.2) | 7 (16.7) | 16 (38.1)*** | 42 |
| PTEN | | | | |
| No deletion | 134 (77.5) | 19 (11.0) | 20 (11.6) | 173 |
| Deletion | 23 (44.2) | 7 (13.5) | 22 (42.3)*** | 52 |
| MET | | | | |
| No aberration | 112 (80.6) | 14 (10.1) | 13 (9.4) | 139 |
| Aberration | 41 (47.1) | 14 (16.1) | 32 (36.8)*** | 87 |

Abbreviations: EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; PTEN, phosphatase and tensin homolog; MET, hepatocyte growth factor receptor.
* $p \leq .05$.
** $p \leq .01$.
*** $p \leq .001$.
‡ Including 2 tumors in Gl. sublingualis.
† Including 2 N3 cases.

TABLE 3. Logistic regression analysis for the presence of lymph node metastasis.

| Variable | Coding | Univariate *P* value* | Multivariate | |
|---|---|---|---|---|
| | | | *P* value | Odds ratio (95% CI) |
| Age | <70 vs >70 | .006 | .733 | 0.84 (0.30–2.35) |
| Grade | G1/G2 vs G3 | < .001 | .262 | 0.47 (0.13–1.75) |
| T classification | 1,2 vs 3,4 | < .001 | **.017** | **3.36 (1.24–9.10)** |
| Histology | | | | |
| SDC | Others vs SDC | < .001 | **.002** | **12.32 (2.44–62.20)** |
| Adenocarcinoma NOS | Others vs adenocarcinoma NOS | .056 | **.047** | **4.18 (1.02–17.15)** |
| SCC | Others vs SCC | .216 | .580 | 1.603 (0.30–8.55) |
| EGFR | No copy number gain vs copy number gain | .004 | .438 | 0.58 (0.14–2.32) |
| HER2 | No copy number gain vs copy number gain | < .001 | .307 | 2.11 (0.50–8.85) |
| PTEN | No Del vs Del | < .001 | **.042** | **3.33 (1.04–10.65)** |
| MET | No Aberr vs Aberr | < .001 | **.009** | **3.81 (1.40–10.42)** |

Abbreviations: CI, confidence interval; SDC, salivary duct carcinoma; NOS, not otherwise specified; SCC, squamous cell carcinoma; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; PTEN, phosphatase and tensin homolog; Del, deletion; MET, hepatocyte growth factor receptor; Aberr, aberration.
* Fisher's exact test.The figures in boldface represent significant predictors.

statistical significance for predicting neck node metastasis in the multivariate analysis.

During the follow-up period, 15 patients (2 ACN, 2 ACC, 2 MEC, 3 SDC, 2 adenocarcinoma NOS, 1 SCC, 1 myoepithelial carcinoma, 1 malignant mixed tumor, and 1 oncocytic carcinoma) developed tumor recurrence as secondary lymph node metastasis (4.8%). A total of 53.8% of these patients (7 of 13) presented aberration of MET ($p = .24$) and 41.7% (5 of 12) deletion of PTEN ($p = .14$). In 13 patients, positive neck nodes occurred despite primary neck dissection. Two (1 low-grade T1 MEC and 1 cribriform T1 ACC, both of the minor salivary glands) of the 51 patients (3.9%) who were initially classified as cN0 and did not undergo neck dissection because of small tumor size and low/intermediate-grade histology developed neck node metastasis (pN1).

## DISCUSSION

Treatment of carcinomas of the major and minor salivary glands is mainly based on surgery, in many cases followed by adjuvant radiotherapy. Neck surgery represents an important component of therapy, because the presence of cervical lymph node metastasis is a major negative predictor of long-term survival.[3,5,6] This negative impact on survival was also confirmed in our analysis, which additionally showed that the current nodal American Joint Committee on Cancer staging procedure implies important differences in survival between patients with N1 and N2 to N3 status. Patients with N1 status still present a 5-year survival rate of 76%, whereas prognosis drops to 34% for patients with N2 or N3 neck node status. Recommendations for the surgical therapy of neck nodes in salivary gland cancer are controversial. Some clinicians recommend treatment of the neck only for patients with clinically and radiographically evident neck node disease. Others recommend neck dissection of the involved side in all major salivary gland cancers,[22] whereas others reserve neck dissection for tumors with certain clinicopathological factors, such as high-grade malignancy or increased tumor size.[5,6,23]

The present analysis showed an incidence of 31.3% for metastatic cervical neck nodes for the total cohort of tumors, which is in line with the incidence rate of 10% to 53% reported in the literature.[5,7,22–25] However, the incidence rate varies depending on several parameters. For example, neck node metastasis occurred in 47% of high-grade malignancies and 43% of T3/T4 tumors but was rarely found in low/intermediate-grade (15%) and T1/T2-classified (23%) tumors. This association is well-known and applies to both major and minor salivary gland tumors.[5,7,26] Advanced patient age was also associated with the presence of neck node metastasis, which can be partly explained by the fact that, for example, SDCs are tumors which often occur in older patients. In our study, SDCs presented neck node metastasis in 71% of the patients, mainly with advanced N2 disease. This percentage shows the exceptional capability of these tumors for cervical lymphatic spread that has already been reported previously.[27–29] Further entities associated with cervical lymph node metastasis in our investigation were adenocarcinomas NOS and primary SCCs. Interestingly, polymorphous low-grade adenocarcinomas also presented positive neck nodes in 30% of cases, which is higher than the 12% to 17% reported in the literature.[30,31] Although nodal disease extension seems limited (pN1 status), elective neck dissection should also be considered for this subtype. In contrast, lymph node metastases were rarely found in ACNs (7%) and ACCs (18%). Reported incidence rates for neck node spread in ACCs are inconsistent, varying between 9% and 38%.[7,22,32,33] In a recent study including 616 ACCs of the major and minor salivary glands, a general incidence rate of 10% was found for cervical lymph node metastasis.[33] In that investigation, neck node metastasis was most frequently found in ACCs located in the tongue (15% to 19%), whereas parotid ACCs showed positive neck nodes in only 3% of cases. In MEC, the probability of neck node metastasis decisively depends on the tumor grade.[34] In our own investigation, high-grade MECs showed positive neck nodes in 47% of cases compared to only 17% for low-grade and intermediate-grade MECs. It has been reported that salivary gland carcinomas originating from the minor glands of the pharynx or larynx more frequently show positive neck nodes (in about 30% to 47%) than those arising in the oral cavity (10% neck node metastases).[7,35]

In the present study, localization of the primary tumor was, however, not indicative for lymph node metastasis, even though we did not differentiate the localization of minor gland disease in further detail.

With view to the results of our multivariate analysis, the histopathological diagnosis of SDC emerged as the strongest clinicopathological predictor of cervical lymph node metastasis (odds ratio, 12.3), followed by the histology of adenocarcinoma NOS and advanced T classification (T3/T4). The importance of primary tumor extension is well known and has been repeatedly described before.[5,7,22,26,32] Surprisingly, according to our classification, the factor "tumor grade'' was not significant for predicting positive neck nodes, although its importance has been mentioned in the literature.[5,26] SDCs have gained importance during the past years as these tumors have been better characterized and thus more properly identified (about 10%) than formerly estimated.[36] In older investigations on neck node predictors, SDCs were most often included into the rubric of adenocarcinomas.[5,7,22,26] However, this subtype is highly suggestive for lymphatic spread and should therefore be considered separately.

Beyond known clinicopathological parameters, we investigated the importance of potential aberrations/amplifications of the EGFR, HER2, and MET receptor tyrosine kinase genes and of the tumor suppressor PTEN, which all have been demonstrated to have strong impact on tumor proliferation, survival, and the metastatic potential in various malignancies.[19,37–39] Moreover, EGFR, HER2, and MET proteins represent established or potential tumor therapy targets.

Increased copy number of genomic EGFR and HER2 has already been previously described in different subtypes of salivary gland cancer. Both EGFR and HER2 gene gain characterize high-grade carcinomas with poor prognosis.[8,40] HER2 is particularly expressed in SDCs.[8,27,41,42] Functionally, HER2 overexpressing tumor cells modulate apoptosis, microenvironment, and cell adhesion molecules (eg, E-cadherin). Therefore, these tumors have the ability to invade through the basement membrane, adhere to endothelial cells, extravasate, and migrate to distant sites.[43] In the current investigation, tumors with an increased copy number of EGFR or HER2 showed lymph node metastasis in 50% and 54% of cases, respectively, compared to the regular EGFR and HER2 status (both 24%). However, both parameters (EGFR and HER2) did not reach significant levels in the multivariate regression analysis.

The receptor tyrosine kinase MET and its ligand, the hepatocyte growth factor, have been found in many solid malignancies, such as non-small cell lung carcinomas, gastric, breast, or head and neck cancer. MET and hepatocyte growth factor activate different cellular signaling pathways leading to tumor cell proliferation, motility, migration, and invasion.[44,45] In salivary gland cancer, aberrations of genomic MET can occur as gene copy number gain (polysomies, amplifications) but also as genomic deletion, and both occurrences are characteristic for high-risk tumors with poor overall survival.[9] Aberration of MET seems to be crucial for lymphatic spread because 53% of the studied salivary gland carcinomas with a MET aberration presented positive neck nodes. Multivariate analysis showed that aberration of genomic MET is a very strong predictor of lymph node metastasis, even stronger than the recognized criteria tumor size and grade. Biologically, MET downstream activation of Gab1, Grb2, PI3K, ERK/MAPK, and other signaling molecules leads to the epithelial mesenchymal transition of epithelial cells, increasing the motility, and disassembly of adhering junctions. Alterations of extracellular matrix proteinases (eg, matrix metalloproteinases), cytoskeletal functions, and cell adhesion molecules (eg, cadherins) enable tumor cells to migrate and form secondary tumors.[45,46]

The tumor suppressor gene PTEN is located on chromosome 10q23 and functions, among others, as a negative regulator of the PI3K/AKT/mTOR signaling axis. Loss of PTEN concurs with the stimulation of the PI3K pathway, thus promoting cell survival and tumor growth.[47] Additionally, loss of PTEN deregulates cell interactions with the extracellular matrix, which leads to integrin-mediated cell spreading and upregulation of the focal adhesion kinase, resulting in cell migration and metastasis.[48] In salivary gland cancer, loss of PTEN has recently been described as hemizygous or homozygous deletions in a subset of highly malignant tumors associated with elevated levels of EGFR and HER2 as well as poor prognosis.[10] The present investigation revealed a significant association between the deletion of genomic PTEN and the occurrence of neck node metastasis. Moreover, in multivariate analysis, loss of PTEN emerged as a strong predictor of lymph node metastasis.

One limitation of our work was that patients with small tumors (T1 and T2) and clinical N0 status who did not undergo neck dissection were grouped together with pathologic N0 patients, and this grouping may include the risk of false-negative diagnosis of neck nodes. Actually, 2 patients with initial cN0 classification (3.9%) developed secondary lymph node metastasis during the follow-up analysis, which suggests a possible false-negative diagnosis of the initial neck node status. On the other hand, the exclusion of patients with small tumors without neck dissection does not seem to be correct either, particularly with view to the multivariate regression analysis for lymph node predictors. If such patients were excluded, the T classification would be biased toward bigger sized tumors and lack the predictive significance between small and large tumors for positive neck nodes.

Occult neck node metastases of salivary gland cancer have been reported in 12% to 45% of cases[5,22,23,25] and in up to 50% of high-grade malignancies.[5] Therefore, establishing predictors of neck node metastasis seems highly important, particularly if clinical or radiological evidence is lacking. This study compares the impact of already established clinicopathological parameters like T classification and histological tumor grade with novel molecular markers, such as EGFR, HER2, MET, and PTEN, on cervical lymph node metastasis. In summary, the histological diagnosis of SDC is the strongest predictor of positive lymph nodes in salivary gland cancer. Elective neck dissection should also be conducted for adenocarcinomas NOS, SCCs, and high-grade MECs as well as for large (T3/T4) tumors. The molecular markers PTEN and MET are powerful predictors of lymph node metastasis, whereas EGFR and HER2 turned out less powerful. PTEN and MET might therefore aid decisions regarding neck dissections in salivary gland cancer.

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## REFERENCES

1. Speight PM, Barrett AW. Salivary gland tumours. *Oral Dis* 2002;8: 229–240.
2. O'Brien CJ, Traynor SJ, McNeil E, McMahon JD, Chaplin JM. The use of clinical criteria alone in the management of the clinically negative neck among patients with squamous cell carcinoma of the oral cavity and oropharynx. *Arch Otolaryngol Head Neck Surg* 2000;126:360–365.
3. Bhattacharyya N, Fried MP. Determinants of survival in parotid gland carcinoma: a population-based study. *Am J Otolaryngol* 2005;26:39–44.
4. Therkildsen MH, Christensen M, Andersen LJ, Schiødt T, Hansen HS. Salivary gland carcinomas–prognostic factors. *Acta Oncol* 1998;37:701–713.
5. Armstrong JG, Harrison LB, Thaler HT, et al. The indications for elective treatment of the neck in cancer of the major salivary glands. *Cancer* 1992; 69:615–619.
6. Medina JE. Neck dissection in the treatment of cancer of major salivary glands. *Otolaryngol Clin North Am* 1998;31:815–822.
7. Lloyd S, Yu JB, Ross DA, Wilson LD, Decker RH. A prognostic index for predicting lymph node metastasis in minor salivary gland cancer. *Int J Radiat Oncol Biol Phys* 2010;76:169–175.
8. Ettl T, Stiegler C, Zeitler K, et al. EGFR, HER2, survivin, and loss of pSTAT3 characterize high-grade malignancy in salivary gland cancer with impact on prognosis. *Hum Pathol* 2012;43:921–931.
9. Ach T, Zeitler K, Schwarz–Furlan S, et al. Aberrations of MET are associated with copy number gain of EGFR and loss of PTEN and predict poor outcome in patients with salivary gland cancer. *Virchows Arch* 2013;462: 65–72.
10. Ettl T, Baader K, Stiegler C, et al. Loss of PTEN is associated with elevated EGFR and HER2 expression and worse prognosis in salivary gland cancer. *Br J Cancer* 2012;106:719–726.
11. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. American Joint Committee on Cancer: AJCC Cancer Staging Manual. 7th ed. New York: Springer; 2010.
12. Barnes L, Eveson JW, Reichart P, Sidransky D. Pathology and genetics of head and neck tumours. World Health Organization Classification of Tumours. Lyon: IARC Press; 2005. p 430.
13. Schwarz S, Stiegler C, Müller M, et al. Salivary gland mucoepidermoid carcinoma is a clinically, morphologically and genetically heterogeneous entity: a clinicopathological study of 40 cases with emphasis on grading, histological variants and presence of the t(11;19) translocation. *Histopathology* 2011;58:557–570.
14. Jouzdani E, Yachouh J, Costes V, et al. Prognostic value of a three-grade classification in primary epithelial parotid carcinoma: result of a histological review from a 20-year experience of total parotidectomy with neck dissection in a single institution. *Eur J Cancer* 2010;46:323–331.
15. Goode RK, El-Naggar AK. Mucoepidermoid carcinoma. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. Pathology and genetics of head and neck tumours. World Health Organization Classification of Tumours. Lyon: IARC Press; 2005. pp 219–220.
16. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;19:403–410.
17. Sassen A, Rochon J, Wild P, et al. Cytogenetic analysis of HER1/EGFR, HER2, HER3 and HER4 in 278 breast cancer patients. *Breast Cancer Res* 2008;10:R2.
18. Cappuzzo F, Ligorio C, Toschi L, et al. EGFR and HER2 gene copy number and response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC). *J Thorac Oncol* 2007;2:423–429.
19. Chung CH, Ely K, McGavran L, et al. Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. *J Clin Oncol* 2006;24:4170–4176.
20. Pectasides E, Rampias T, Kountourakis P, et al. Comparative prognostic value of epidermal growth factor quantitative protein expression compared with FISH for head and neck squamous cell carcinoma. *Clin Cancer Res* 2011;17:2947–2954.
21. Sircar K, Yoshimoto M, Monzon FA, et al. PTEN genomic deletion is associated with p-Akt and AR signalling in poorer outcome, hormone refractory prostate cancer. *J Pathol* 2009;218:505–513.
22. Stennert E, Kisner D, Jungehuelsing M, et al. High incidence of lymph node metastasis in major salivary gland cancer. *Arch Otolaryngol Head Neck Surg* 2003;129:720–723.
23. Rodríguez–Cuevas S, Labastida S, Baena L, Gallegos F. Risk of nodal metastases from malignant salivary gland tumors related to tumor size and grade of malignancy. *Eur Arch Otorhinolaryngol* 1995;252:139–142.
24. Spiro RH. Salivary neoplasms: overview of a 35-year experience with 2,807 patients. *Head Neck Surg* 1986;8:177–184.
25. Zbären P, Schüpbach J, Nuyens M, Stauffer E, Greiner R, Häusler R. Carcinoma of the parotid gland. *Am J Surg* 2003;186:57–62.
26. Bhattacharyya N, Fried MP. Nodal metastasis in major salivary gland cancer: predictive factors and effects on survival. *Arch Otolaryngol Head Neck Surg* 2002;128:904–908.
27. Jaehne M, Roeser K, Jaekel T, Schepers JD, Albert N, Löning T. Clinical and immunohistologic typing of salivary duct carcinoma: a report of 50 cases. *Cancer* 2005;103:2526–2533.
28. Ettl T, Schwarz S, Kleinsasser N, Hartmann A, Reichert TE, Driemel O. Overexpression of EGFR and absence of C-KIT expression correlate with poor prognosis in salivary gland carcinomas. *Histopathology* 2008;53: 567–577.
29. Lewis JE, McKinney BC, Weiland LH, Ferreiro JA, Olsen KD. Salivary duct carcinoma. Clinicopathologic and immunohistochemical review of 26 cases. *Cancer* 1996;77:223–230.
30. Seethala RR, Johnson JT, Barnes EL, Myers EN. Polymorphous low-grade adenocarcinoma: the University of Pittsburgh experience. *Arch Otolaryngol Head Neck Surg* 2010;136:385–392.
31. Perez–Ordonez B, Linkov I, Huvos AG. Polymorphous low-grade adenocarcinoma of minor salivary glands: a study of 17 cases with emphasis on cell differentiation. *Histopathology* 1998;32:521–529.
32. Régis De Brito Santos I, Kowalski LP, Cavalcante De Araujo V, Flávia Logullo A, Magrin J. Multivariate analysis of risk factors for neck metastases in surgically treated parotid carcinomas. *Arch Otolaryngol Head Neck Surg* 2001;127:56–60.
33. Min R, Siyi L, Wenjun Y, et al. Salivary gland adenoid cystic carcinoma with cervical lymph node metastasis: a preliminary study of 62 cases. *Int J Oral Maxillofac Surg* 2012;41:952–957.
34. McHugh CH, Roberts DB, El-Naggar AK, et al. Prognostic factors in mucoepidermoid carcinoma of the salivary glands. *Cancer* 2012;118: 3928–3936.
35. Schramm VL Jr, Imola MJ. Management of nasopharyngeal salivary gland malignancy. *Laryngoscope* 2001;111:1533–1544.
36. Brandwein–Gensler MS, Skalova A, Nagao T. Salivary duct carcinoma. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. Pathology and genetics of head and neck tumours. WHO Classification of Tumours, volume 9. Lyon: IARC Press; 2005. pp 236–237.
37. Lehrer S, Lee P, Tartter P, Brower S. Tumour HER2 protein in breast cancer and family history. *Lancet* 1993;341:1420–1421.
38. Cappuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J Clin Oncol* 2009;27:1667–1674.
39. Yoshimoto M, Cunha IW, Coudry RA, et al. FISH analysis of 107 prostate cancers shows that PTEN genomic deletion is associated with poor clinical outcome. *Br J Cancer* 2007;97:678–685.
40. Lujan B, Hakim S, Moyano S, et al. Activation of the EGFR/ERK pathway in high-grade mucoepidermoid carcinomas of the salivary glands. *Br J Cancer* 2010;103:510–516.
41. Williams MD, Roberts DB, Kies MS, Mao L, Weber RS, El-Naggar AK. Genetic and expression analysis of HER-2 and EGFR genes in salivary duct carcinoma: empirical and therapeutic significance. *Clin Cancer Res* 2010;16:2266–2274.
42. Skálová A, Stárek, Kucerová V, Szépe P, Plank L. Salivary duct carcinoma–a highly aggressive salivary gland tumor with HER-2/neu oncoprotein overexpression. *Pathol Res Pract* 2001;197:621–626.
43. Freudenberg JA, Wang Q, Katsumata M, Drebin J, Nagatomo I, Greene MI. The role of HER2 in early breast cancer metastasis and the origins of resistance to HER2-targeted therapies. *Exp Mol Pathol* 2009;87:1–11.
44. Appleman LJ. MET signaling pathway: a rational target for cancer therapy. *J Clin Oncol* 2011;29:4837–4838.
45. Benvenuti S, Comoglio PM. The MET receptor tyrosine kinase in invasion and metastasis. *J Cell Physiol* 2007;213:316–325.
46. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;4:915–925.
47. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol* 2010;28:1075–1083.
48. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science* 1998;280:1614–1617.