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Clinicopathological characteristics and outcome of 31 patients with *ETV6-NTRK3* fusion gene confirmed (mammary analogue) secretory carcinoma of salivary glands

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ABSTRACT

Objectives: In 2010, a new subtype of salivary gland cancer (SGC), (mammary analogue) secretory carcinoma (SC), was defined, characterized by the *ETV6-NTRK3* fusion gene. As clinical behavior and outcome data of this histological subtype tumor are still sparse, we aimed to describe the clinicopathological course and outcome of a series of translocation positive SC patients.

Patient and methods: We re-evaluated the pathological diagnosis of a subset of SGCs, diagnosed in 4 of 8 Dutch head and neck centers. Subsequently, tumors with a morphological resemblance to SC were tested for the *ETV6-NTRK3* fusion gene using RT-PCR. Furthermore, patients prospectively diagnosed with SC were included. The clinical characteristics and outcomes were retrieved from the patient files.

Results: Thirty-one patients with ETV6-NTRK3 fusion gene positive SC were included. The median age was 49 years, 17 patients (55%) were male. Eighteen tumors (58%) arose in the parotid gland. One patient presented with lymph node metastasis. All patients underwent tumor resection and 4 patients had a neck dissection. Four patients had re-resection and 15 patients (48%) received postoperative radiotherapy. One patient developed a local recurrence, no regional recurrences or distant metastases were observed. After a median follow-up of 49 months the 5- and 10-year overall survival were 95%, the 5- and 10-year disease free survival were 89%.

Conclusion: The clinical course of SC is favorable with a low rate of locoregional recurrence and excellent survival. Given the low incidence of nodal metastases, elective neck treatment, i.e. surgery and/or radiotherapy, does not seem to be indicated.

Introduction

Salivary gland cancers comprise a wide histological spectrum with more than twenty different subtypes [1]. In 2010, a new entity of salivary gland cancer was described by Skálová et al., characterized by the presence of the *ETV6-NTRK3* fusion gene [2]. The histopathological appearance resembles secretory carcinoma of the breast, and both tumors share the *ETV6-NTRK3* fusion gene, hence the proposed name was mammary analogue secretory carcinoma (MASC). In the updated 2017 WHO classification, MASC is acknowledged and referred to as 'secretory carcinoma', to standardize nomenclature amongst different organ sites [1].

The morphological features of secretory carcinoma include a variety of architectural growth patterns, intracytoplasmic vacuoles, lack of intracytoplasmic zymogen granules in a mucinous or hemosiderin-laden histiocyte-rich background [3]. Immunohistochemical markers such as S100, vimentin, STAT5a, MUC4 and mammaglobin may be helpful in preselecting patients with suspected secretory carcinoma, but none of these markers can fully confirm the diagnosis. However, the presence of the *ETV6-NTRK3* fusion gene is pathognomonic [4]. The

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most important entities in the differential diagnosis of secretory carcinoma are acinic cell carcinoma (AciCC), polymorphous adenocarcinoma (PAC), and adenocarcinoma not otherwise specified (NOS). Although in salivary gland cancer the presence of the fusion gene is specific for secretory carcinoma, the *ETV6-NTRK3* fusion gene has also been demonstrated in several other solid and hematological malignancies, such as secretory carcinoma of the breast, papillary thyroid carcinoma, congenital fibrosarcoma, congenital mesoblastic nephroma, acute myeloid leukemia and sino-nasal low-grade adenocarcinoma [5–8].

In recent years, several reports have been published on characteristic histopathological features of secretory carcinoma within the many subtypes of SGC, but little is known about the clinical behavior of this new entity, including the outcome of these patients. A review of 279 cases showed a male to female ratio of 1.5:1 and occurrence mostly (68%) in the parotid gland [9]. Disease free survival (DFS) in secretory carcinoma patients was reported to be similar to DFS in AciCC in a comparison in respectively 29 and 38 patients [10]. The aim of the current study is to focus on clinical behavior and outcome of patients with secretory carcinoma.

Patients and methods

In four hospitals in the Netherlands, patients with subsets of salivary gland cancer (AciCC, PAC and adenocarcinoma) were retrospectively evaluated for morphological resemblance to secretory carcinoma by pathologists (U.F, L.S, E.B. and S.M.W.) from 2000 until 2016. Patients suspected of secretory carcinoma were tested for the presence of the *ETV6-NTRK3* fusion gene. The *ETV6-NTRK3* fusion gene was analyzed using reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was extracted from formalin-fixed and paraffin-embedded tissues (FFPE) using RNA-Bee-RNA isolation reagent (Bio-Connect BV, Huissen, the Netherlands) according to standard procedures. RNA quantity and quality were determined by NanoDrop measurement (Fisher Scientific, Landsmeer, the Netherlands) and, subsequently, cDNA synthesis was performed using Superscript II (Invitrogen Life Technologies Europe, Bleiswijk, the Netherlands) and random hexamers (Promega Nederland, Leiden, the Netherlands).

The cDNA was tested by the reverse transcription-polymerase chain reaction (RT-PCR) for the *HMBS* (hydroxymethylbilase synthase) housekeeping gene using the primers forw150 5′-TGCCAGAGAAGAGT GTGGTG-3′, rev150 5′-ATGATGGCACTGAACTCCTG-3′, forw250 5′-CTGGTAACGGCAATGCGGCT-3′, rev250 5′- TTCTTCTCCAGGGCATG TTC-3′.

For detection of the *ETV6-NTRK3* fusion, the following primers were used: *ETV6* forward primer P385: 5′-ACCACATCATGGTCTCTGTCT CCC-3′and *NTRK3* reverse primer P386: 5′-CAGTTCTCGCTTCAGCAC GATG-3′. The PCR products were analyzed by agarose gel electrophoresis.

Patients who were prospectively diagnosed with secretory carcinoma were also included. For both retrospective and prospective cases, the presence of the *ETV6-NTRK3* fusion gene was mandatory for inclusion in this study. Patients' characteristics regarding clinical presentation, diagnosis, treatment and follow-up were collected by evaluating medical records. According to the Dutch guidelines, review by a medical ethical committee was not necessary due to the retrospective nature of this study (www.federa.org).

Resection margins were categorized as free (> 5 mm), close (1–5 mm) or involved (< 1 mm) based on the pathology reports. For further survival analysis, close and involved margins were grouped as 'not free'.

For the prospectively collected cases the date of diagnosis was defined as first date of histopathological confirmation of the diagnosis secretory carcinoma. In retrospective cases, the date of obtaining the original histopathological material was used as date of diagnosis.

Statistics

Overall survival (OS) is defined as the time from date of diagnosis until date of death of any cause. Patients alive at the last known follow-up date were censored. DFS is defined as the time from date of surgery until date of recurrence (local or regional recurrence or distant metastasis) or death of any cause, whichever comes first. Patients alive without disease recurrence at last known follow-up were included in the analysis as censored. OS and DFS were estimated using Kaplan Meier survival curves. Statistical analysis was performed using SPSS data analysis software version 22.0.

Results

Patients and tumor characteristics

In total, 42 patients were tested; 3 patients tested negative, for 6 patients the fusion gene could neither be confirmed nor invalidated, in 33 patients we confirmed the presence of the *ETV6-NTRK3* fusion gene. Unfortunately, clinical records were not available for 2 of the 33 confirmed patients; thus, a total of 31 patients with *ETV6-NTRK3* fusion gene positive secretory carcinoma were included. The median age at diagnosis was 49 years (range 19–83 years), 17 patients (55%) were male. Eighteen tumors (58%) were located in the parotid gland, one in the submandibular gland, and the remaining 12 tumors in the minor salivary glands. The primary site of the minor salivary gland tumors were the lip (n = 5), the oral mucosa (n = 2), the soft palate (n = 1), the hard palate (n = 1) and the remaining three could not be further specified. Nineteen patients (61%) had T1 tumors, 10 patients (32%) T2 tumors; for 2 patients the T-classification was not available. Only one patient presented with regional lymph node metastasis, none of the patients had distant metastasis at diagnosis. In most patients, the presenting symptom was a painless mass (22 patients). One patient presented with a painful swelling and one with a non-healing wound. For the remaining 9 patients no symptoms could be retrieved from the medical records. The median time from start of symptoms to diagnosis was 14 months (range 6 weeks–20 years).

Eleven patients were prospectively diagnosed with secretory carcinoma between 2011 and 2016. The remaining 20 patients were retrospectively diagnosed with secretory carcinoma, confirmed by the presence of the *ETV6-NTRK3* fusion gene. The initial diagnosis of these patients was between 2000 and 2012. Of these 20 patients, 16 patients were previously diagnosed with AciCC, three as PAC and one as adenocarcinoma NOS.

Baseline characteristics of all patients are shown in Table 1.

Primary treatment

Primary treatment for all 31 patients consisted of surgery. Seventeen patients had a surgical resection of the affected salivary gland, and in four of them surgery also included a neck dissection (only one patient had tumor positive lymph nodes). Eleven patients had either a local excision, excision or incision biopsy of the tumor. For three patients, the exact type of surgery could not be determined. Seven patients (23%) had involved resection margins, nine patients had closely excised tumors (29%) and 13 (42%) had free resection margins. In two patients, information about resection margins was not available. Four patients (13%) had additional surgery, because of involved resection margins (2), a close excision margin (1) and an uncertain margin after excision biopsy. Fifteen patients (48%) received postoperative radiotherapy: all seven patients with involved resection margins, six out of nine patients with closely excised tumors and two patients with free resection margins. One of the patients with free resection margins had a difficult preparation of the facial nerve during surgery, and was therefore treated with postoperative radiotherapy. For the other patient with free resection margins the exact reason for