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Fine needle aspiration of salivary gland carcinomas with high-grade transformation: A multi-institutional study of 22 cases and review of the literature

Running title:
Salivary Gland Carcinoma FNA with HGT

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AUTHORS' CONTRIBUTIONS

All authors of this article declare that we qualify for authorship as defined by *Cancer Cytopathology*. Each author has participated sufficiently in the work and takes public responsibility for appropriate portions of the content of this article. MN carried out the background research and drafted the manuscript. MN, WCF, and PMS conceived the idea for the manuscript and its design and coordination. MN, WCF, ZWB, RLC, MLC, KAE, BJH, RU, DAK, KTM, MN, LP, EDR and PMS were involved in collation of cases. MN, WCF, ZWB, RLC, MLC, KAE, BJH, RU, DAK, KTM, MN, LP, EDR and PMS participated revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

ABSTRACT

BACKGROUND: High-grade transformation (HGT) is a rare process whereby conventional low- to intermediate-grade salivary gland carcinomas (SGC) transform into high-grade, poorly or undifferentiated malignancies with focal or complete loss of their conventional histomorphologic features. Because HGT is associated with a worse prognosis than their conventional counterparts, preoperative recognition of HGT may be of benefit for optimal patient management. Using a multi-institutional approach, we describe the largest FNA cohort of salivary gland carcinomas with HGT.

METHODS: The archives of 9 large academic medical centers were searched, and 22 cases of SGC with HGT were identified by surgical excision accompanied by preoperative FNA. Clinical and cytomorphologic features were retrospectively reviewed.

RESULTS: The male-to-female ratio was 14:8, and the mean patient age was 60.2 years. The average tumor size was 3.6 cm, and 19 cases were from the parotid gland. Acinic cell carcinoma with HGT was the most common tumor subtype, comprising 12 cases with HGT, followed by adenoid cystic carcinoma, secretory carcinoma, and other subtypes. Eighteen cases were classified as malignant; however, a specific diagnosis of HGT was not made. Sixteen cases contained a high-grade cytologic component, and 7 cases had a mixture of both conventional and high-grade components retrospectively.

CONCLUSIONS: SGC with HGT should be considered in the differential diagnosis of a salivary gland aspirate exhibiting high-grade cytomorphologic features. The presence of distinct tumor populations, conventional and high-grade, should prompt consideration of HGT, especially when the conventional component is acinic cell carcinoma or adenoid cystic carcinoma.

INTRODUCTION

Primary salivary gland carcinoma (SGC) is classified in the most recent *WHO Classification of Head and Neck Tumours* into twenty-one tumor types.[1, 2] While salivary duct carcinoma is an inherently high-grade, aggressive carcinoma,[3] most subtypes of SGC are known to be biologically low- to intermediate-grade. However, these lower grade SGC can rarely transform into high-grade malignancies associated with marked cellular atypia, hyperchromasia, and increased mitotic activity. This phenomenon is termed high-grade transformation (HGT), and greater than 100 cases, mostly as case reports and small series, involving acinic cell carcinoma (AcCC), adenoid cystic carcinoma (AdCC), and several other tumor types have been reported in the literature.[4-7] The transformed component presents histologically as a poorly differentiated or undifferentiated carcinoma with at least partial loss of the original characteristic histomorphologic phenotype.[4, 5, 7]

From a clinical perspective, HGT is known to impart more aggressive biologic behavior and a worse prognosis than conventional low- to intermediate-grade SGC.[4, 6-8] Preoperative recognition of a high-grade component by fine needle aspiration (FNA) would therefore be useful for patient prognostication as well as for influencing patient management, including presurgical planning.[9] Despite wide recognition of this disease concept, only a few case reports describing the cytological features of SGC with HGT have been published,[10-17] and comprehensive FNA case series are lacking. Using a multi-institutional approach, we describe the clinical and cytomorphologic features of the largest FNA series of SGC with HGT and provide a comprehensive review of the literature.

MATERIAL AND METHODS

The institutional review boards of each collaborating institution approved the current study. We performed a retrospective search of the institutional pathology archives of nine large academic medical centers (Massachusetts General Hospital (Boston, MA), Stanford University Medical Center (Palo Alto, CA), Hospital of the University of Pennsylvania (Philadelphia, PA), University of Wisconsin School of Medicine and Public Health (Madison, WI), Beth Israel Deaconess Medical Center (Boston, MA), Dartmouth-Hitchcock Medical Center (Lebanon, NH), Vanderbilt University Medical Center (Nashville, TN), Agostino

Gemelli School of Medicine (Rome, Italy), and University of Michigan-Michigan Medicine (Ann Arbor, MI)) from 1997 to 2020 for SGC with HGT and corresponding FNA material. The surgical pathology slides of the tumors were reviewed by three head and neck pathologists (MN, PMS, and WCF), and cases fulfilling the histomorphologic criteria for SGC with HGT as defined in the previous studies and by the WHO[4, 6, 7, 18-22] were included in the study cohort. Twenty-two tumors from 22 patients with HGT on excision with preoperative FNA met the inclusion criteria. Sixteen of the cohort cases were from primary salivary gland tumors, and 6 FNA cases were from recurrent lesions with a documented primary tumor, all from distinct patients. The diagnosis of HGT was established either in the primary tumor excision specimen or in tissue from tumor recurrence or metastasis. The FNA diagnostic classifications were based on data from the original cytopathology reports adjusted to the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).[2] Twenty of the FNA cohort cases with slides available for review were examined by study authors and evaluated for a series of cytomorphologic features including cellular cytomorphology, FNA cellularity, nuclear pleomorphism, nucleoli, nuclear/cytoplasmic ratio, and necrotic background. For the remaining two cases, information for diagnostic category and cytological diagnoses were collected from cytology reports.

RESULTS

Clinical and Pathologic Findings

In total, 22 tumors from 22 patients with HGT noted on excision with preoperative FNA were enrolled in this study. Clinical characteristics for the cohort of FNA cases are summarized in Table 1. The patients' age ranged from 24 to 82 years (mean, 60.2 years) with a male:female ratio of 14:8. Tumors ranged in size from 1.8 to 6.2 cm (average size: 3.6 cm). Six FNA samples were obtained from recurrent or metastatic sites, including cervical or mediastinal lymph nodes, lung, and stylomastoid foramen. Eighty-six percent of cases (n=19) originated in the parotid gland, while the submandibular gland, hard palate, and maxillary sinus together contributed the remaining 14% of primary case sites (n=3). The most common original conventional tumor type was AcCC (n=12, 55%), followed by AdCC (n=5, 23%), secretory carcinoma (n=2, 9%), epithelial-myoepithelial carcinoma, mucoepidermoid carcinoma, and clear cell carcinoma (n=1, 4.5% each). A high-grade component comprised more than 50% of tumor areas in the corresponding surgical resection specimen in 50% of the cases (n=11, 7 primary and 4 recurrent), >10% to 50% of the tumor in 32% (n=7, 5 primary and 2 recurrent), and 0 to 10% of the tumor in 18% (n=4, 4 primary) of the cohort cases. Among cases with

available clinical treatment and follow-up data, all patients received post-operative chemotherapy and/or radiotherapy, and 64% of patients (n=14) experienced cancer recurrence or metastasis. Twenty-seven percent of the patients were alive without disease (n=6), 32% were alive with disease (n=7), and 41% (n=9) died of the disease with follow-up periods of 0.8 to 126 months (median, 8.8 months).

Cytomorphologic Findings

Cytomorphologic findings of the FNA cohort cases are summarized in Table 2 and corresponding representative cytologic features are shown in Figs. 1 and 2. The FNA from the primary tumor (n=16) was classified as malignant in 75% (n=12) of cases, followed by salivary gland neoplasm of uncertain malignant potential (SUMP) in 19% of cases (n=3), and suspicious for malignancy in a single case (6.3%) by the MSRSGC.[2] By original report, among 22 FNA, 32% (n=7) were designated as high-grade, but a specific diagnosis of HGT was not suggested. The definitive cytologic diagnosis of a conventional tumor subtype was rendered in 3 cases regardless of the tumor grading; one recurrent secretory carcinoma, one AcCC diagnosed by immunohistochemistry using the cell block material, and one AdCC with characteristic cytomorphologic features of AdCC. The diagnoses of the other FNA were descriptive without specifying tumor subtypes.

In the retrospective slide evaluation, 85% of the FNA cohort was moderately or highly cellular (n=17). All aspirates (100%, n=20) contained cohesive groups of neoplastic cells with or without a component of isolated cells. Thirty-five percent (n=7, 5 primary, 2 recurrent) of HGT cases contained cells representing both the conventional lower grade tumor and high-grade component (Fig. 1). In contrast, 45% of HGT FNA (n=9, 5 primary, 4 recurrent) were composed of only cells from the high-grade component (Figs. 2A, B), and 20% (n=4, 4 primary) of cases contained neoplastic cells only from the conventional lower grade component (Figs. 2 C, D). In total, 80% (n=16) of cases contained cells from the high-grade component. Tumor cells representing HGT were disorganized, less cohesive and had larger and more pleomorphic nuclei with conspicuous nucleoli and occasional mitoses compared to the lower grade conventional SGC component. Among the HGT component, nuclear pleomorphism, prominent nucleoli, high nuclear/cytoplasmic ratio, and necrotic background were present in 60% (n=12), 55% (n=11), 90% (n=18), and 40% (n=8) of the FNA cases, respectively. Cell blocks were prepared in 73% of cases (16/22 cases), and ancillary studies were performed in 32% of the total FNA cases (7/22 cases) including immunohistochemical stains (n=7) and fluorescence in situ hybridization (n=3).

DISCUSSION

FNA is considered an effective and informative method for the preoperative evaluation of salivary gland tumors. It is useful not only in helping to differentiate malignant tumors from benign tumors, but also for distinguishing high-grade carcinomas from those that are low-grade, guiding treatment management.[23] HGT is histologically characterized by the transformation of a conventional carcinoma (low- to intermediate-grade) into a high-grade, poorly differentiated or undifferentiated carcinoma.[4, 5] Based upon published reports, the most common SGC subtypes in cases of HGT are AcCC and AdCC.[5-7] In our FNA cohort, AcCC was the predominant HGT-associated tumor subtype followed by AdCC. In this sense, aspirates of cases identified as AcCC or AdCC with unusual higher grade features should prompt consideration that the case might represent HGT and warrant comment in the cytologic report.

The cytomorphologic findings of HGT in previously published reports are summarized in Table 3.[10-17] Among 8 single case reports, all FNA specimens, except for one report of metastatic SGC with HGT in pleural effusion cytology,[17] were from primary salivary gland tumors.[10-16] All reported FNA specimens were diagnosed as malignant. Retrospective analysis of the reported cases revealed two cell components, the lower grade conventional tumor and the component with HGT, in 6 cases, solely cells from HGT component in 1 case, and solely from the conventional tumor component in 1 case. Cytological diagnosis of high-grade or poorly differentiated carcinoma were made in 6 cases, and HGT was suggested in 2 cases, including 1 recurrent case.

Although HGT itself is associated with a poor clinical prognosis,[4, 6, 7, 21] there is no data regarding what percentage of high-grade component influences patient prognosis. In our cohort, the proportion of HGT component determined on the excision specimen varied from those with a limited HGT component to those that were almost exclusively composed of a HGT component (Table 1). Although our cohort of primary tumors is limited (n=16), in terms of overall cohort size and length of follow-up, and although the true clinical significance should bear out in follow-up studies with an expanded cohort, the relationship between clinical outcomes and the proportion of the high-grade component in the primary tumor resection specimen is presented and summarized in Table 4. Patients with primary tumors with a lower proportion of high-grade component tended to have lower recurrence/metastasis rate and lower overall mortality with a median follow up of just 6.8 months. We present these data as part of the discussion as an intriguing consideration to include percentage of HGT on surgical

resections as a possible prognostic indicator.

Certainly, the extent of the HGT component in the FNA specimen would be expected to influence the diagnostic accuracy of the FNA, at least in terms of grading the lesion. Thirty-five percent of cases in our cohort (n=7) and 6 of 8 FNA cases in previous reports contained both tumor components (conventional tumor and HGT; Table 3). On the other hand, 20% of cases in our cohort (n=4) and 1 of 8 case in previous reports contained only the conventional low- to intermediate-grade component of SGC, which represents a sampling-related pitfall for identifying the carcinoma as high-grade preoperatively. The proportion of HGT component in these 4 excision cases in our study were ≤10% in 2 cases and >10% to 50% in 2 cases. While specific interpretation of HGT may seem elusive, this entity should be included in the differential diagnosis of a high-grade SGC, especially for those cases comprised of 2 distinct epithelial components- a conventional low- to intermediate-grade type admixed with a high-grade subpopulation.

The differential diagnosis of HGT includes salivary duct carcinoma, high-grade mucoepidermoid carcinoma, a subset of myoepithelial carcinomas, adenocarcinoma NOS, and metastatic cancers from other anatomic sites. From a clinical perspective, it is more important for management purposes that the FNA identifies the neoplasm as high-grade than it is to give a specific diagnosis of HGT.[24, 25] In our cohort with available FNA slides, a high-grade component was detected in 80% of cases (16/20 cases). Although the distinction is usually of less clinical importance except perhaps for metastatic carcinomas, the distinction of HGT cases from salivary duct carcinoma may influence the pre- and post-operative management including the use of androgen receptor- and HER2- targeted therapy.[3] Abundant eosinophilic cytoplasm and immunoexpression of androgen receptor, GATA3, GCDFP-15, and HER2 using cell block material can assist in distinguishing salivary duct carcinoma from SGC with HGT.[3] The specific diagnosis of a conventional tumor component, especially AcCC or AdCC, was given in 3 of our cohort cases using ancillary studies performed on cell block material. Although the diagnosis of a conventional tumor subtype may not be necessary, ancillary immunohistochemistry or fluorescence in situ hybridization can help to establish the diagnosis of a primary SGC and exclude the possibility of metastasis.[26, 27]

Given that many of the conventional SGC are characterized by specific genetic alterations, the ancillary studies on cell block material are useful to establish the diagnosis.[26, 28] Based on the discovery of an *NR4A3* translocation in AcCC,[29] a recent study demonstrated

the usefulness of *NR4A3* FISH and NR4A3 IHC in FNA specimens.[27] *MYB*, *NTRK3*, *MAML2*, and *EWSR1* FISH might have facilitated a diagnosis of AdCC, secretory carcinoma, mucoepidermoid carcinoma, or clear cell carcinoma in our FNA cohort.[26] The definitive diagnosis of conventional carcinoma with a high-grade component would suggest the possibility of HGT. While we did not investigate the molecular features of our FNA cohort, future studies exploring the molecular biology of SGC with HGT may prove fruitful, and perhaps, such studies would reveal a molecular signature for HGT that would be applied to FNA cases.

In conclusion, SGC with HGT should be considered in the differential diagnosis of salivary gland aspirates of high-grade carcinomas, especially when the high-grade component has an undifferentiated appearance. The presence of 2 distinct cellular components (a conventional tumor component and high-grade component) should prompt consideration for HGT, especially when the conventional component is AcCC or AdCC, and, importantly, it should also prompt consideration of a diagnostic comment about this possibility in the cytology report.

REFERENCES

1. El-Naggar AK. WHO classification of head and neck tumours. International Agency for Research on Cancer, 2017.
2. Faquin WC, Rossi ED, Baloch Z, et al. The Milan system for reporting salivary gland cytopathology. Springer, 2018.
3. Nakaguro M, Tada Y, Faquin WC, Sadow PM, Wirth LJ, Nagao T. Salivary Duct Carcinoma: Updates in Histology, Cytology, Molecular Biology, and Treatment. Cancer Cytopathol. 2020.
4. Nagao T. "Dedifferentiation" and high-grade transformation in salivary gland carcinomas. Head Neck Pathol. 2013;7 Suppl 1: S37-47.
5. Nakaguro M, Faquin WC, Sadow PM. Cytologic and Histologic Aspects of High-Grade Transformation of Salivary Gland Carcinoma. AJSP: Reviews & Reports. 2020;25: 243-248.
6. Seethala RR, Hunt JL, Baloch ZW, Livolsi VA, Leon Barnes E. Adenoid cystic carcinoma with high-grade transformation: a report of 11 cases and a review of the literature. Am J Surg Pathol. 2007;31: 1683-1694.
7. Thompson LD, Aslam MN, Stall JN, Udager AM, Chiosea S, McHugh JB. Clinicopathologic and Immunophenotypic Characterization of 25 Cases of Acinic Cell

Carcinoma with High-Grade Transformation. Head Neck Pathol. 2016;10: 152-160.
8. Petersson F. High-Grade Transformation (“Dedifferentiation”)—Malignant Progression of Salivary Gland Neoplasms, Including Carcinoma ex Pleomorphic Adenoma: A Review. AJSP: Reviews & Reports. 2015;20: 27-37.
9. Johnson DN, Onenerk M, Krane JF, et al. Cytologic grading of primary malignant salivary gland tumors: A blinded review by an international panel. Cancer Cytopathol. 2020;128: 392-402.
10. González-Peramato P, Jiménez-Heffernan JA, López-Ferrer P, Vicandi B, Viguer JM. Fine needle aspiration cytology of dedifferentiated acinic cell carcinoma of the parotid gland: a case report. Acta Cytol. 2006;50: 105-108.
11. Malhotra KP, Agrawal V, Pandey R. High grade transformation in adenoid cystic carcinoma of the parotid: report of a case with cytologic, histologic and immunohistochemical study. Head Neck Pathol. 2009;3: 310-314.
12. Johnykutty S, Miller CH, Hoda RS, Giampoli EJ. Fine-needle aspiration of dedifferentiated acinic cell carcinoma: Report of a case with cyto-histological correlation. Diagn Cytopathol. 2009;37: 763-768.
13. Suzuki T, Murata S, Yamaguchi H, Shimizu Y, Shimizu M. Epithelial-myoepithelial carcinoma with myoepithelial anaplasia: report of a case with cytologic findings of a rare variant. Acta Cytol. 2010;54: 605-610.
14. Jung MJ, Kim SY, Nam SY, et al. Aspiration cytology of mammary analogue secretory carcinoma of the salivary gland. Diagn Cytopathol. 2015;43: 287-293.
15. Bhardwaj M, Gupta P. Dedifferentiated adenoid cystic carcinoma ex pleomorphic adenoma of the parotid. J Cancer Res Ther. 2018;14: 706-708.
16. Dutta A, Arun P, Arun I. Adenoid Cystic Carcinoma with Transformation to High Grade Carcinomatous and Sarcomatoid Components: A Rare Case Report with Review of Literature. Head Neck Pathol. 2020.
17. Pusztaszeri MP, Brochu V. Metastatic adenoid cystic carcinoma with high-grade transformation ("dedifferentiation") in pleural effusion and neck lymph node: A diagnostic challenge on cytology? Diagn Cytopathol. 2020;48: 679-683.
18. Nagao T, Gaffey TA, Kay PA, et al. Dedifferentiation in low-grade mucoepidermoid carcinoma of the parotid gland. Hum Pathol. 2003;34: 1068-1072.
19. Skálová A, Sima R, Vanecek T, et al. Acinic cell carcinoma with high-grade transformation: a report of 9 cases with immunohistochemical study and analysis of TP53 and HER-2/neu genes. Am J Surg Pathol. 2009;33: 1137-1145.

20. Skálová A, Vanecek T, Majewska H, et al. Mammary analogue secretory carcinoma of salivary glands with high-grade transformation: report of 3 cases with the ETV6-NTRK3 gene fusion and analysis of TP53, β-catenin, EGFR, and CCND1 genes. Am J Surg Pathol. 2014;38: 23-33.
21. Roy P, Bullock MJ, Perez-Ordoñez B, Dardick I, Weinreb I. Epithelial-myoepithelial carcinoma with high grade transformation. Am J Surg Pathol. 2010;34: 1258-1265.
22. Jin R, Craddock KJ, Irish JC, Perez-Ordonez B, Weinreb I. Recurrent hyalinizing clear cell carcinoma of the base of tongue with high-grade transformation and EWSR1 gene rearrangement by FISH. Head Neck Pathol. 2012;6: 389-394.
23. Cibas ES, Ducatman BS. Cytology: Diagnostic Principles and Clinical Correlates. 5th edition ed. Philadelphia: Elsevier, 2020.
24. Fang Q, Wu J, Du W, Zhang X. Predictors of distant metastasis in parotid acinic cell carcinoma. BMC Cancer. 2019;19: 475.
25. Seethala RR, Altemani A, Ferris RL, et al. Data Set for the Reporting of Carcinomas of the Major Salivary Glands: Explanations and Recommendations of the Guidelines From the International Collaboration on Cancer Reporting. Arch Pathol Lab Med. 2019;143: 578-586.
26. Jo VY, Krane JF. Ancillary testing in salivary gland cytology: A practical guide. Cancer Cytopathol. 2018;126 Suppl 8: 627-642.
27. Skaugen JM, Seethala RR, Chiosea SI, Landau MS. Evaluation of NR4A3 immunohistochemistry (IHC) and fluorescence in situ hybridization and comparison with DOG1 IHC for FNA diagnosis of acinic cell carcinoma. Cancer Cytopathol. 2020.
28. Bishop JA. Immunohistochemistry surrogates for molecular alterations: A new paradigm in salivary gland tumor cytopathology? Cancer Cytopathol. 2020.
29. Haller F, Bieg M, Will R, et al. Enhancer hijacking activates oncogenic transcription factor NR4A3 in acinic cell carcinomas of the salivary glands. Nat Commun. 2019;10: 368.

FIGURE LEGENDS

Figure 1. Cytologic features of high-grade transformation (HGT) exhibiting 2-cell patterns. (A) FNA of acinic cell carcinoma with HGT. Tumor cells from a high-grade component (arrow) have larger nuclei and form disorganized cellular clusters compared to the conventional

component (arrowhead) (Papanicolaou stain). (B) Histologic features of the same case (H & E stain). The tumor loses features of acinic cell carcinoma (left panel) and transforms into poorly differentiated carcinoma (right panel). (C, D) Higher magnification of conventional and high-grade components of acinic cell carcinoma with HGT (Papanicolaou stain). Tightly cohesive clusters of uniform basaloid cells with small nuclei suggestive of low-grade carcinoma (C). Large pleomorphic cells with occasional prominent nucleoli form less cohesive clusters or are present as single cells (D). (E) Diff-Quik stain of secretory carcinoma with HGT. 2 cell populations with different sizes and chromatin patterns are observed. (F) Cell block specimen of an FNA of acinic cell carcinoma with HGT. Clear contrast between two components is demonstrated. Mitosis is noted in the high-grade component (H & E stain).

Figure 2. Cytologic features of high-grade transformation (HGT) with one component. (A, B) FNA of HGT showing solely the high-grade carcinoma component (Papanicolaou stain). (A) FNA of adenoid cystic carcinoma with HGT. Large pleomorphic are present in clusters with nuclear crowding. Inflammatory cells and necrotic debris are noted. (B) FNA of acinic cell carcinoma with HGT. The original acinic cell differentiation is totally lost and the cytologic features are indistinguishable from (A). (C, D) FNA of adenoid cystic carcinoma with HGT showing only the conventional carcinoma component. (C) Basaloid hyperchromatic cells are present around matrix globules. This case was signed out as adenoid cystic carcinoma (Diff-Quik stain). (D) Cohesive clusters occasionally associated with matrix-like material. Although slight nuclear size variation is noted, no obvious high-grade features are observed (Papanicolaou stain).

TABLE 1. Clinical and Pathologic Characteristics of Salivary Gland Carcinoma with High-Grade Transformation

Gender (n [%])	Male	14 (64)
	Female	8 (36)
Age (mean [range]) (y)		60.2 (24-82)
Tumor size (mean [range]) (cm)		3.6 (1.8-6.2)
Original tumor site	Parotid	19 (86)
	Submandibular	1 (4.5)
	Hard palate	1 (4.5)
	Maxillary sinus	1 (4.5)
Histology	AcCC-HGT	12 (55)
	AdCC-HGT	5 (23)
	SC-HGT	2 (9.0)
	EMC-HGT	1 (4.5)
	MEC-HGT	1 (4.5)
	CCC-HGT	1 (4.5)
High-grade component	0-10%	4 (18) (4 primary)
	>10-50%	7 (32) (5 primary, 2 recurrence)
	>50-100%	11 (50) (7 primary, 4 recurrence)
Post-operative therapy	Chemotherapy	1 (4.5)
	Radiation	8 (36)
	Chemotherapy+ radiation	13 (59)

Follow-up (median [range]) (mo)		8.8 (0.8-126)
Recurrence or Metastasis	Yes	14 (64)
	No	8 (36)
Patient outcome	Alive without disease	6 (27, 3.8)
(n, [%, median follow-up]) (mo)	Alive with disease	7 (32, 19.7)
	Dead with disease	9 (41, 10.7)

Abbreviations: AcCC, acinic cell carcinoma; AdCC, adenoid cystic carcinoma; CCC, clear cell carcinoma; EMC, epithelial-myoepithelial carcinoma; HGT, high-grade transformation; MEC, mucoepidermoid carcinoma; SC, secretory carcinoma.

TABLE 2. Cytologic Characteristics of Salivary Gland Carcinoma with High-Grade Transformation

		n (%)
Diagnostic category of FNA from primary site (N=16)	Malignant	12 (75)
	Suspicious for malignancy	1 (6.3)
	Neoplasm: SUMP	3 (19)
Diagnostic category of FNA from recurrent site (N=6)	Malignant	6 (100)
Tumor grading (N=22)	High	7 (32)
	Intermediate to high	1 (4.5)
	Intermediate	2 (9)
	Low to intermediate	1 (4.5)
	Low	1 (4.5)
	Not graded	10 (45)
Preoperative diagnosis of HGT (N=22)	Yes	0(0)
	No	22 (100)
Specific diagnosis of tumor subtype (N=22)	Yes	3 (14)
	No	19 (86)
FNA cellularity (N=20)	Low	3 (15)
	Moderate	2 (10)
	High	15 (75)
Groups or single cells (N=20)	Groups	7 (35)
	Single cells	0 (0)
	Both	13 (65)

Cellular component (N=20)	LG+HG	7 (35) (5 primary, 2 recurrent)
	HG only	9 (45) (5 primary, 4 recurrent)
	LG only	4 (20) (4 primary)
Nuclear pleomorphism (N=20)	Present	12 (60)
	Absent	8 (40)
Nucleoli (N=20)	Prominent	11 (55)
	Small or inconspicuous	9 (45)
High nuclear/cytoplasmic ratio (N=20)	Present	18 (90)
	Absent	2 (10)
Necrotic background (N=20)	Present	8 (40)
	Single cell necrosis/ inflammatory	3 (15)
	Absent	9 (45)

Abbreviations: HG, high-grade component; LG, conventional lower-grade component; SUMP, salivary gland neoplasm of uncertain malignant potential

TABLE 3. Cytologic features of Salivary Gland Carcinoma with High-Grade Transformation in Previous Studies

	Histology	Diagnostic category	Cytologic diagnosis	Cell component	Nuclear pleomorphism	Prominent nucleoli	High nuclear/ cytoplasmic ratio	Background
González-Peramato et al.	AcCC-HGT	Malignant	Salivary carcinoma, AcCC and HG carcinoma	LG+HG	Present	Present	Present	Lymphoid
Malhotra et al.	AdCC-HGT	Malignant	Adenocarcinoma, NOS, Undifferentiated carcinoma	LG+HG	Present	Present	Present	Hemorrhagic
Johnykutty et al.	AcCC-HGT	Malignant	High-grade salivary gland neoplasm	LG+HG	Present	Present	Present	NS
Suzuki et al.	EMC-HGT	Malignant	NS	LG+HG	Present	Present	NS	NS
Jung et al.	SC-HGT	Malignant	NS	HG only	Present	Present	Present	Clear
Bhardwaj et al.	AdCC-HGT	Malignant	Poorly diff. carcinoma	LG+HG	Present	Present	NS	Necrotic, myxoid matrix
Dutta et al.	AdCC-HGT	Malignant	Basaloid neoplasm, AdCC/ basal cell adenocarcinoma	Presumably LG only	Absent	Absent	Present	NS
Pusztaszeri et al.	AdCC-HGT	Malignant	AdCC-HGT, consistent with	LG+HG	Present	Present	NS	Histiocytic, lymphoid

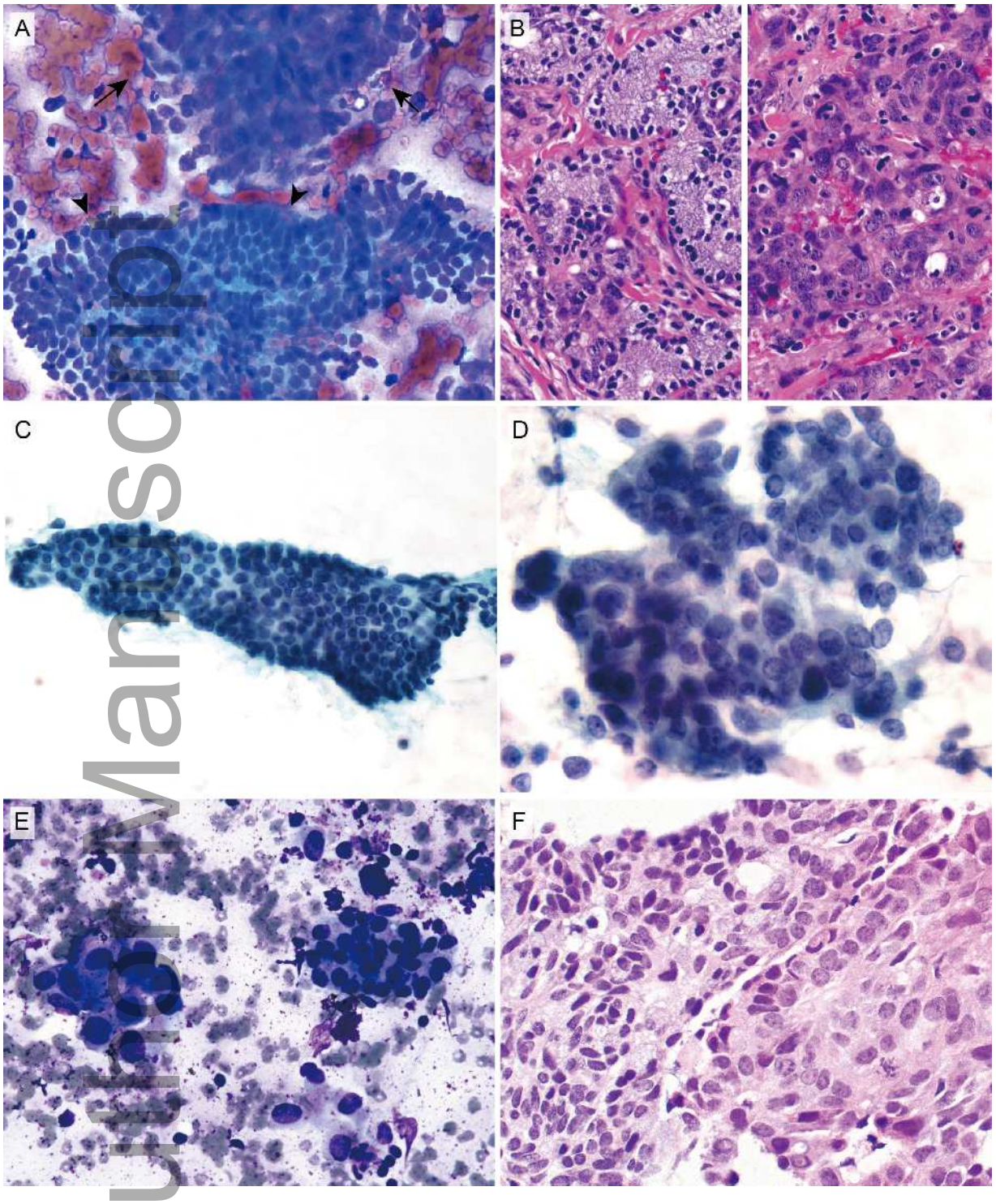
Abbreviations: AcCC, acinic cell carcinoma; AdCC, adenoid cystic carcinoma; EMC, epithelial-myoepithelial carcinoma; HG, high-grade component; HGT, high-grade transformation; LG, conventional lower grade component; NS, not stated; SC, secretory carcinoma.

TABLE 4. Clinical Outcomes and Proportion of a High-Grade Component in The Primary Tumor (N=16)

High-grade component	Recurrence or metastasis		Patient outcome		
	Yes	No	Alive without disease	Alive with disease	Dead with disease
0-10%	1	3	3	0	1
>10-50%	2	3	2	1	2
>50-100%	5	2	1	3	3

Median follow-up was 6.8 months.

The proportion of high-grade component was calculated on the resection specimen.



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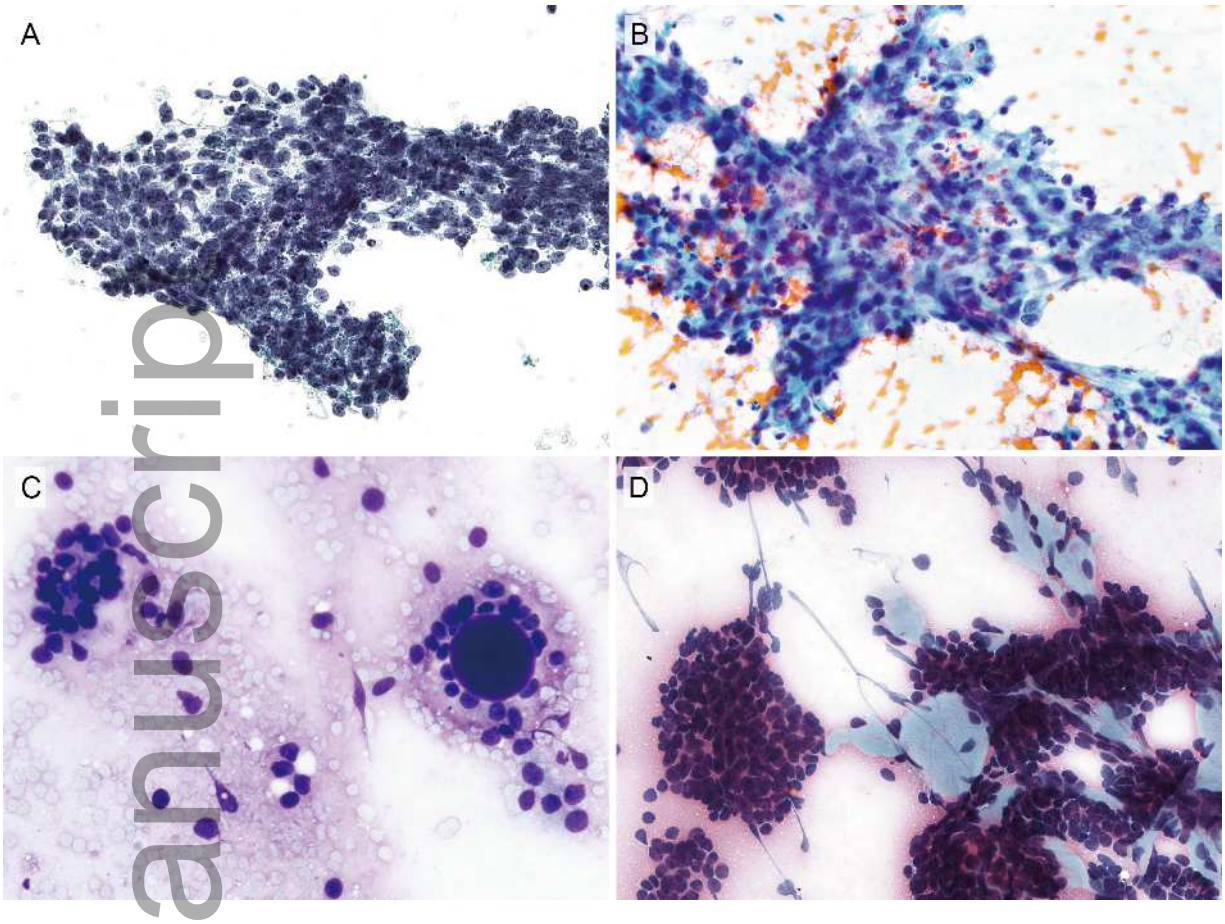

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