

Cytokeratin immunoprofile of primary and metastatic adenoid cystic carcinoma of salivary glands: a report of two cases

Cibele Pidorodeski Nagano^a, Cláudia Malheiros Coutinho-Camillo^b, Clovis Antônio Pinto^b, Fernando Augusto Soares^{a,b}, Filipa Santos^c, Isabel Fonseca^c, Silvia Vanessa Lourenço^a

Nagano CP, Coutinho-Camillo CM, Pinto CA, et al. Cytokeratin immunoprofile of primary and metastatic adenoid cystic carcinoma of salivary glands: a report of two cases. *Autopsy Case Rep* [Internet]. 2016;6(4):57-63. <http://dx.doi.org/10.4322/acr.2016.056>

ABSTRACT

Distant metastases from salivary gland tumors are considered infrequent: the incidence of distant metastases ranges from 24% to 61% according to different histotypes and to the site of the primary mass. The most common site of distant metastases due to salivary gland malignancies is the lung. From the pathology point of view, cytokeratins (CK) are important differentiation markers in salivary gland tumors, which are often used for the diagnostic process. Their employment also may be useful to identify and confirm the diagnosis of their distant metastases. We report the expression of CK in two cases of primary and metastatic adenoid cystic carcinoma (ACC) and their CK profiles of the primary and metastatic masses. Both patients—one male and one female—were diagnosed with an ACC cribriform and tubular, respectively, with lung metastases. In case 1, the metastatic mass presented the same histotype and CK profile of the primary tumor. For case 2, the metastatic lung mass was distinct from the primary mass (a solid ACC) and presented a different CK profile. Although salivary gland metastatic disease presents a poor prognosis, both patients reported herein are alive despite the presence of the disease in long-term follow-up. Therefore, the modifications seen in the CK profiles do not appear to be predictive of tumor behavior and outcome. The use of a CK profile seems to be useful to identify the nature of a distant mass and its possible correlations with a primary salivary gland tumor.

Keywords

Salivary Gland Neoplasms; Neoplasm Metastasis; Keratins; Carcinoma, Adenoid Cystic.

INTRODUCTION

Salivary gland neoplasms are uncommon, and according to the World Health Organization (WHO), they comprise around 40 histological types. Their etiology is unknown, and the only well-established risk factor is ionizing radiation.¹

The most common salivary gland malignant neoplasms are mucoepidermoid carcinoma and adenoid cystic carcinoma, and the incidence of these entities varies according to different series studied.¹ Both neoplasms are more common in females.

^a Department of Stomatology - Dental School - Universidade de São Paulo, São Paulo/SP – Brazil.

^b Department of Surgical Pathology - AC Camargo Cancer Center, São Paulo/SP – Brazil.

^c Instituto Português de Oncologia - Universidade de Lisboa, Lisboa – Portugal.



They have persistent growth and a tendency to metastasize; high-grade tumors grow rapidly and are destructive and symptomatic.^{1,2}

Histopathological diagnosis of salivary gland tumors is, in many cases, challenging, and depends on the understanding of the typical structures of the organ. Salivary gland neoplasms may arise from the different regions of the organ. The glands are composed of structures formed by luminal cells (acinar and ductal cells) and abluminal cells (myoepithelial and basal cells) (Figure 1). These cells exhibit a well-known antigen profile of cytoskeleton proteins, among others, and this profile can help to identify tumor histogenesis, and therefore a better classification.³ The origin of mucoepidermoid carcinoma is reputed to be the excretory duct cells of salivary glands; it is composed of mucous, and intermediate and epidermoid cells. The origin of adenoid cystic carcinoma (ACC) is regarded to be the intercalated duct cells, and is composed of ductal and myoepithelial cells in tubular, cribriform, and solid architectures.¹

Distant metastasis from salivary gland tumors is considered to be infrequent. The incidence of distant metastasis varies according to different histotypes and the primary location. The most common site of distant metastasis due to salivary gland malignancies

is the lung; the literature reports that 75-90% of all patients with distant metastasis showed pulmonary involvement. However, metastatic masses from salivary gland neoplasms have been reported also in bone, liver, and lymph nodes.²

We report two cases of primary and metastatic ACC with their cytokeratin (CK) profiles (Ethics Committee CAAE: 07907812.8. 1001.0075).

CASE 1

A 42-year-old male presented a history of an operated ACC of the palate. The surgery comprised an extensive maxillectomy up to the infratemporal and pterygopalatine fossae. Histopathology revealed a cribriform ACC with perineural infiltration and angiolymphatic emboli (Figure 2A and 2B). The patient received radiotherapy at 45 Gy. After a 5-year follow-up, two masses (measuring 10 mm and 6 mm at their longest axis) were detected in the inferior lobule of the right lung, which was excised and diagnosed as metastatic salivary gland cribriform ACC (Figure 2C).

The expression of CK7 was detected in luminal structures that intermingled neoplastic myoepithelial cells in cribriform islands of the primary ACC (Figure 3A and 3B). Neoplastic luminal structures

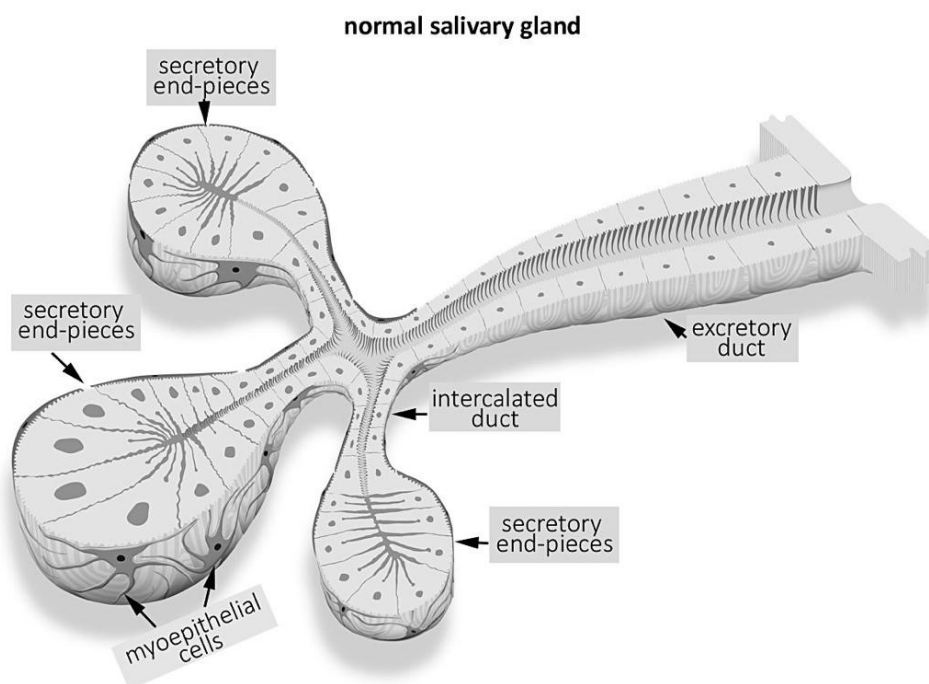


Figure 1. Schematic view of the normal salivary gland structure showing the secretory end-pieces composed of acinar cells surrounded by myoepithelial cells. The intercalated ducts are formed by luminal cells and are surrounded by myoepithelial cells. The excretory ducts are composed of luminal and abluminal cells.

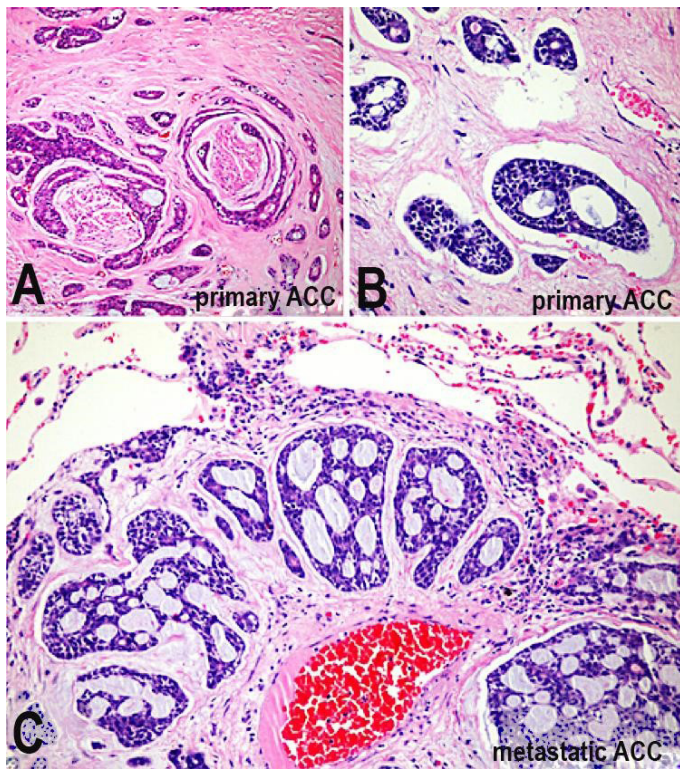


Figure 2. Photomicrography of the histopathological aspects of primary and metastatic adenoid cystic carcinoma. **A** and **B** - Primary cribriform adenoid cystic carcinoma (ACC) of the hard palate. **A** - Cribriform aspect of the primary mass composed of epithelial (luminal) cells and myoepithelial (abluminal) cells around a nerve bundle (perineural infiltration); **B** - Cribriform ACC: angiolympathic spread (emboli) (both H&E, 250X); **C** - Lung metastatic mass from a salivary gland ACC: cribriform aspects of the metastatic mass intermingling the lung parenchyma (H&E, 250X).

expressed CK7 only focally in the lung metastasis (Figures 3C and 3D).

CK14 was focally in the primary ACC, mainly in the luminal structures (Figures 4A, 4B). In the lung mass (4C and 4D), the neoplastic cells were diffusely positive for CK14. The expression of CK18 and CK19 was detected in the luminal structures of the primary mass (Figures 5A, 5B, 6A, and 6B). In the lung mass, expression of CK18 was only focally detected in the luminal structures that were distant from the interface of the lung parenchyma (Figures 5C and 5D). CK19 was focally positive in neoplastic luminal structures of the metastatic mass (Figures 6C and 6D).

The patient was further treated with radiation at 30 Gy and is alive with the disease in a 10-year follow-up.

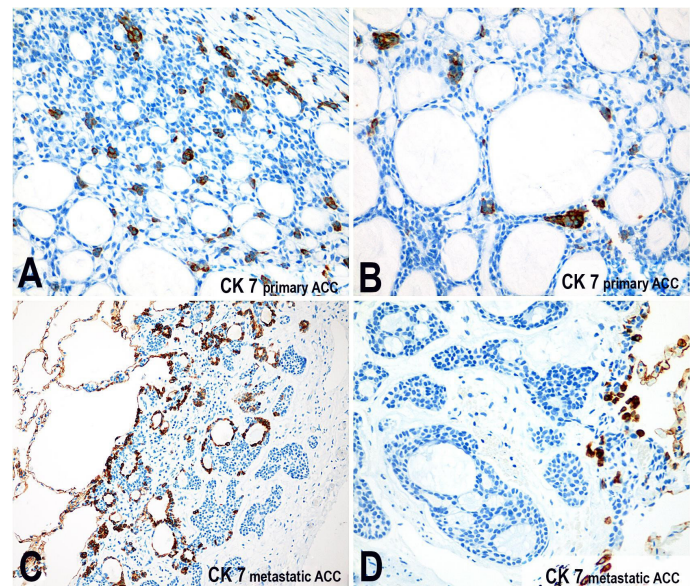


Figure 3. Photomicrography of the expression of CK7 (clone LL002, AbCAM) in the primary and metastatic adenoid cystic carcinoma (ACC). In **A** and **B**, the luminal cells were positive for CK7 in the primary mass; **C** - Metastatic ACC showing a few neoplastic structures positive for CK7; **D** - The tumor islands close to the lung parenchyma are mainly negative for CK7, contrasting with the epithelial lining of the pulmonary alveoli that express this cytokeratin. Immunohistochemistry was revealed with DAB (200X, 400X, 100X, and 200X, respectively).

CASE 2

A 66-year-old female presented a mass on the hard palate, which at histopathology was diagnosed as a cribriform ACC with tubular areas. The tumor recurred locally after 8 years, with similar histopathological aspects; however perineural invasion and angiolympathic spread were not detected. The recurrent lesion was treated with a maxillectomy and radiotherapy at 60 Gy. After 5 years of follow-up, a mass (measuring 8 mm at its longest axis) was found in the right lung. The lesion was surgically treated and diagnosed as a metastasis of a salivary gland ACC (cribriform with solid areas). The histopathological aspects of the primary mass and the metastatic lung mass are illustrated in Figures 7A and 7B.

Expression of CK7 was detected in the tubular structures of the primary palatal mass (Figures 8A and 8B). CK7 was focally expressed within the solid metastatic mass (Figures 8C and 8D). CK14 was positive in the primary tumor (Figures 9A and 9B).

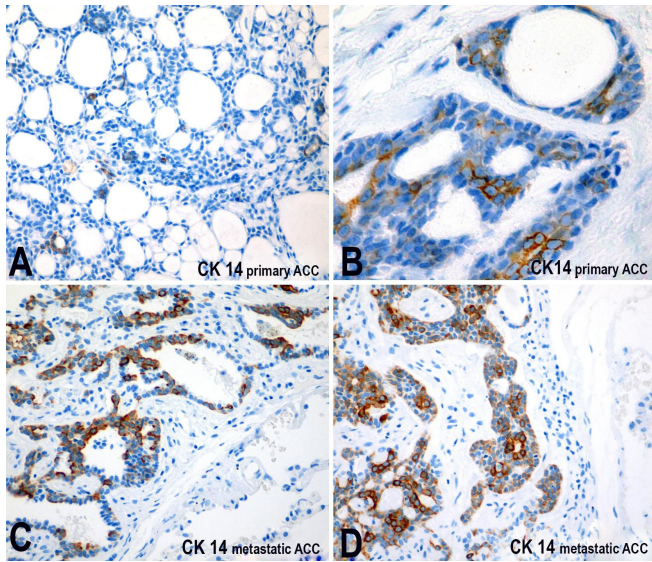


Figure 4. Photomicrography of the expression of CK14 (clone RCK105, AbCAM) in the primary and metastatic adenoid cystic carcinoma (ACC). **A** - A few luminal structures positive for CK14 in the primary ACC; **B** - Cribriform nest of the primary mass showing a greater number of luminal structures positive for CK14. In the metastatic specimens (**C** and **D**) the majority of neoplastic cells are positive for CK14; In **C**, the epithelial lining of the pulmonary alveoli is also positive for this marker. Immunohistochemistry revealed with DAB (all figures 200X).

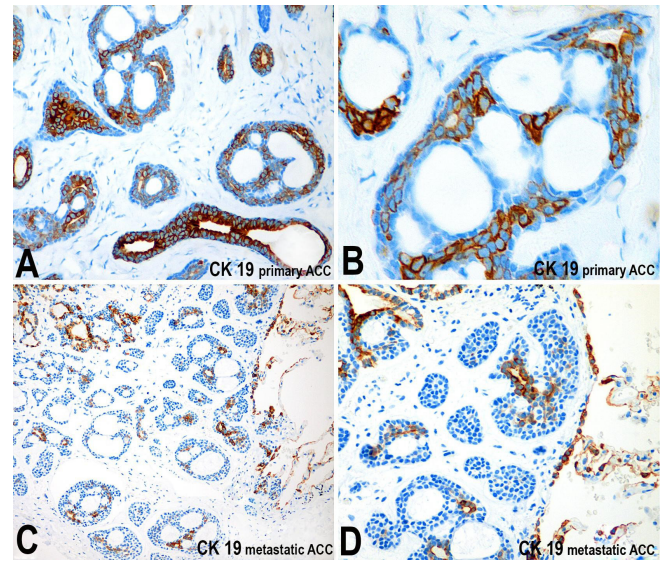


Figure 6. Photomicrography of the expression of cytokeratin 19 (clone A53B/A2, AbCAM) in the primary and metastatic adenoid cystic carcinoma (ACC). **A** and **B** - In the primary mass, luminal cells are highly positive for CK19; **C** and **D** - Representations of the metastatic lung mass show similar patterns with luminal cells positive for CK19. The epithelial lining of the lung is also positive for this protein. Immunohistochemistry revealed with DAB (200X, 400X, 100X, and 200X, respectively).

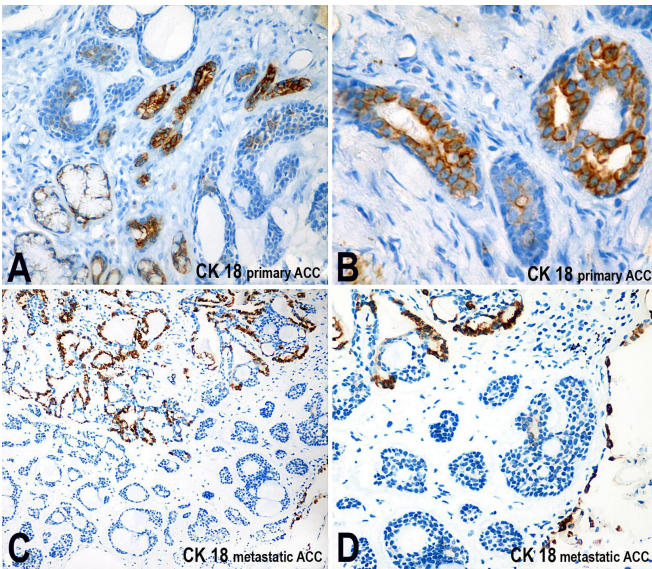


Figure 5. Photomicrography of the expression of CK18 (clone C-04, AbCAM) in the primary and metastatic adenoid cystic carcinoma (ACC). In the primary mass, the luminal cells are highly positive for CK18 (**A** and **B**). **C** and **D** reveal that only neoplastic structures of the metastatic mass, distant from the lung parenchyma interface show luminal cells positive for CK18. The epithelial lining of the lung is positive for this protein. Immunohistochemistry revealed with DAB (200X, 400X, 100X, and 200X, respectively).

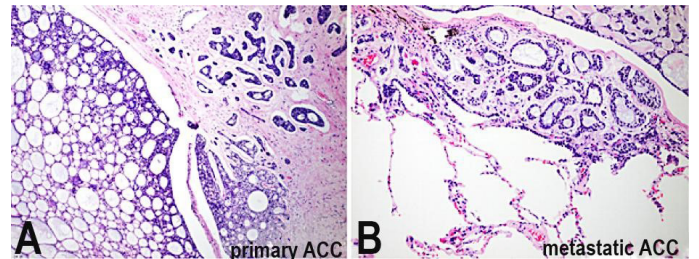


Figure 7. Photomicrography of the histopathological aspects of primary and metastatic adenoid cystic carcinoma ACC. **A** - Primary tubular ACC of the hard palate. Cribriform aspects of the primary mass with marked vascular emboli H&E, 100X; **B** - Lung metastasis of the palatal ACC of the palate. Cribriform area infiltrating lung alveoli (H&E, 100X).

The lung mass was mainly negative for CK14, except for focal positive areas (Figure 9C). Only focal positivity was observed for CK18 both in the primary ACC and in the metastatic mass (Figures 10A-10D). CK19 was expressed by luminal cells in the primary mass (Figures 11A and 11B), but only focal areas were positive in the lung metastasis (Figure 11C).

The patient is alive with disease after a 3-year follow-up. The immunoprofile of both cases is

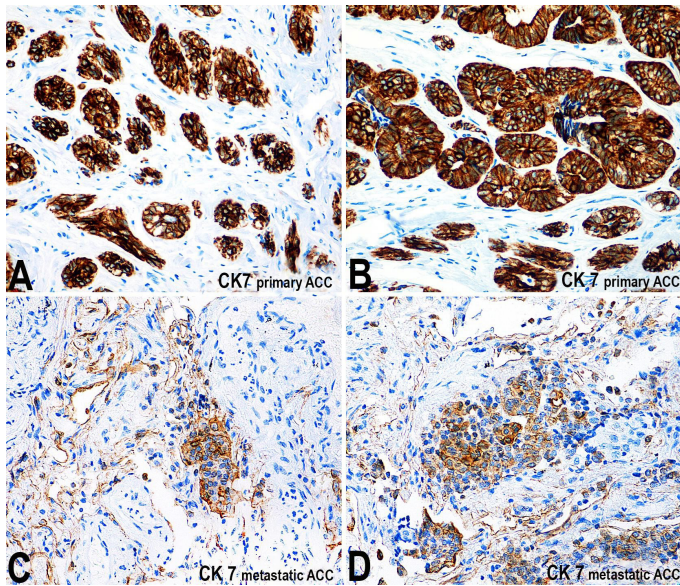


Figure 8. Photomicrography of the expression of CK7 (clone LL002, AbCAM) in the primary and metastatic adenoid cystic carcinoma (ACC). **A** and **B** - Luminal cells are positive for CK7 in the primary tubular ACC; **C** and **D** - Metastatic ACC showing a few neoplastic cells positive for CK7 within the solid tumor mass. Immunohistochemistry revealed with DAB (100X, 250X, 100X, and 200X, respectively).

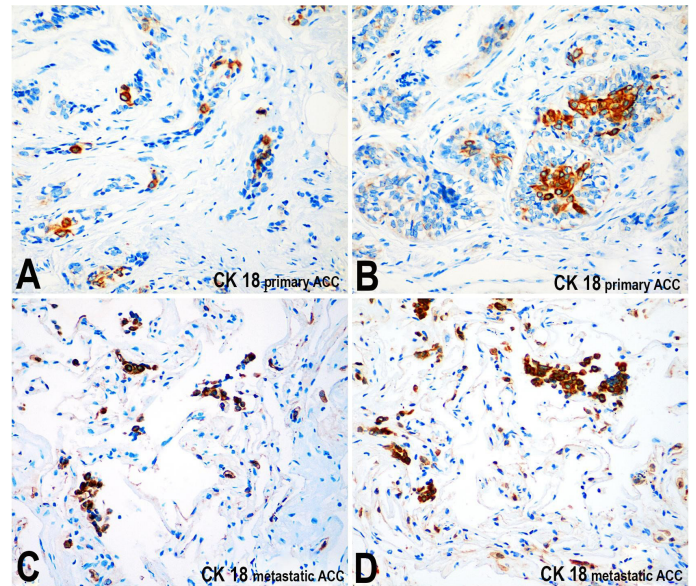


Figure 10. Photomicrography of the expression of CK18 (clone C-04, AbCAM) in the primary and metastatic adenoid cystic carcinoma (ACC). **A** and **B** - In the primary mass, scattered cells are highly positive for CK18; **C** and **D** - Small neoplastic nests within the metastatic mass are positive for CK18. Immunohistochemistry revealed with DAB (100X, 200X, 100X, and 200X, respectively).

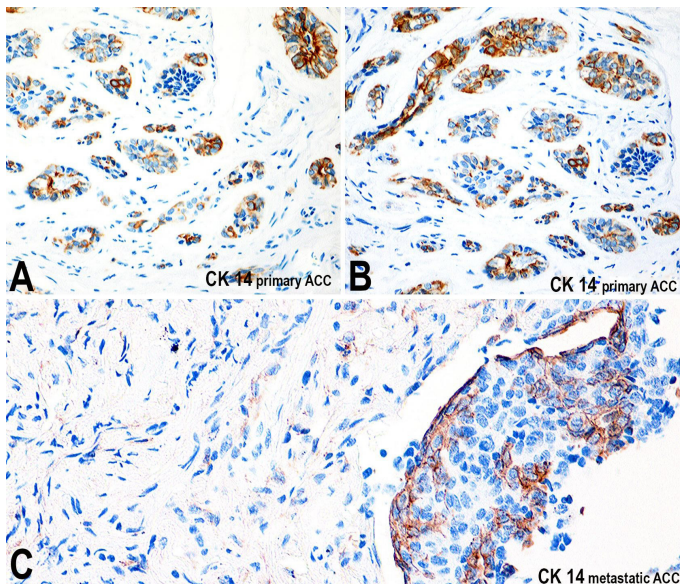


Figure 9. Photomicrography of the expression of CK14 (clone RCK105, AbCAM) in the primary and metastatic adenoid cystic carcinoma (ACC). **A** and **B** - Luminal cells positive for CK14 in the primary ACC; **C** - A rare area positive for CK14 in the solid metastatic mass of the lung. Immunohistochemistry revealed with DAB (100X, 200X, and 400X, respectively).

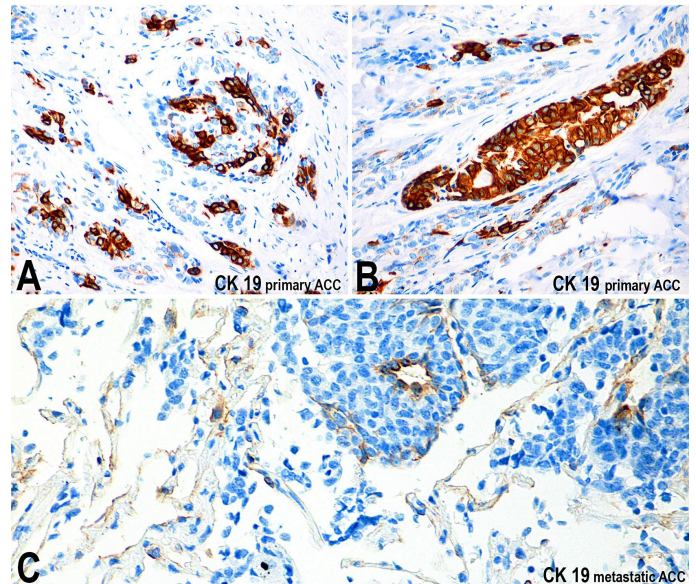


Figure 11. Photomicrography of the expression of cytokeratin 19 (clone A53B/A2, AbCAM) in the primary and metastatic adenoid cystic carcinoma. **A** and **B** - In the primary mass, luminal cells are strongly positive for CK19; **C** - Only a few areas are positive for this protein within the solid metastatic mass. Immunohistochemistry revealed with DAB (100X, 250X, and 400X, respectively).

Table 1. Cytokeratins (CK) immunoprofile of primary and metastatic adenoid cystic carcinoma

	Histological type	CK7	CK14	CK18	CK19	
Case 1	Primary mass	Cribriform	+ve (Luminal structures)	+ve (Luminal structures)	+ve (Luminal structures)	
	Lung metastasis	Cribriform	-ve (Luminal structures); focally positive in scattered cells	+ve (Diffusely within the metastatic mass)	+ve (Focal)	+ve (Focal)
Case 2	Primary mass	Cribriform with tubular areas	+ve (Tubular structures)	+ve (Luminal cells)	+ve (Focal)	+ve (Luminal structures)
	Lung metastasis	Cribriform with solid areas	+ve (Focal)	-ve (Luminal structures); +ve (focal)	+ve (Focal)	Mainly negative

+ve: positive; -ve: negative.

summarized in Table 1, which depicts the major differences between primary and metastatic masses.

DISCUSSION

ACC is one of the most common salivary gland malignancies.² The cases reported herein were of distinct histotypes, which have both arisen from minor salivary glands of the palate. The patients, one male and one female, developed lung metastasis of different ages. According to Bradley,² age and gender are not associated with the development of salivary gland tumor metastasis. However, a large cohort of the Memorial Sloan Kettering Cancer Center Hospital reports that metastasis from salivary gland malignant tumors is more common in men, who are usually diagnosed at an advanced clinical stage.⁴

The development of distant metastases from salivary gland tumors depends on four main factors: (i) the site of the lesion; (ii) the size and duration of the disease at the time of the initial diagnosis; (iii) histotype; and (iv) tumor stage. Tumors over 3 cm are highly predictable of distant metastases.^{2,4} In our cases, the performed mutilating treatment modality (maxillectomy) may have been indicative of large tumors.

The WHO⁵ defines ACC as a basaloid tumor consisting of epithelial and myoepithelial cells with varied architecture. The solid histotype has a relentless clinical course and a usually fatal outcome, and is more likely to develop distant metastases. Other types of

ACC, such as tubular and cribriform, also may develop metastases as shown herein.

The histopathological analysis of the primary mass and metastases is critical for a patient's overall evaluation. As seen in the presented cases, the metastatic mass may or may not follow the architectural patterns of the primary mass. The histopathological findings of the primary mass and lung metastases from patient 1 presented a predominant cribriform pattern. However, the primary mass exhibited a dominant tubular pattern in patient 2, but the lung metastasis was less differentiated with a solid morphology.

Immunohistochemistry enhances diagnostic accuracy as it aids in unraveling the architecture of primary tumors and distant metastases. ACC is consistently positive for cytokeratins AE1/3, 34betaE12, CK5/6, CK7, CK14, and CK18, amongst other markers.³ In our results, the CK expression of the metastatic tumor showed marked changes compared with the primary tumor, mainly with the loss of CK expression. This diversity was observed in case 2, which showed a marked phenotype change in the metastatic mass. This was revealed not only by its morphological aspects but also by the cytokeratin profile.

Salivary gland tumors are virtually incurable, and their metastases presents a poor prognosis. Intriguingly, however, our patients are still alive with present evidence of new metastatic nodules in the lungs of both patients. Alterations in CK profiles do not appear to be predictive of tumor behavior and clinical outcome. Modern molecular techniques, such

as detection of genetic changes like the translocations with the resulting fusion of the *MYB* oncogene, are used in large centers as reliable biomarkers of distant metastases.⁵⁻⁷

ACKNOWLEDGEMENTS

The authors are thankful to Mr. Fabio Andriolo for the design of Figure 1 and for the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)* for sponsoring Cibele Pidorodeski Nagano scholarship.

REFERENCES

1. World Health Organization (WHO). International Agency for Research on Cancer (IARC). Tumours of the Salivary glands: World Health Organization Classification of tumours: pathology and Genetics of head and neck tumours. Lyon: IARC Press; 2005.
2. Bradley PJ. Distant metastases from salivary glands cancer. *J Otorhinolaryngol Relat Spec.* 2001;63(4):233-42. PMID:11408820. <http://dx.doi.org/10.1159/000055748>.
3. Namboodiripad A. A review: immunological markers for malignant salivary gland tumours. *J Oral Biol Craniofac Res.* 2014;4(2):127-34. PMID:25737930. <http://dx.doi.org/10.1016/j.jobcr.2014.05.003>.
4. Ali S, Bryant R, Palmer FL, et al. Distant metastasis in patients with carcinoma of the major salivary glands. *Ann Surg Oncol.* 2015;22(12):4014-9. PMID:25743328. <http://dx.doi.org/10.1245/s10434-015-4454-y>.
5. Ettl T, Schwarz-Furlan S, Gosau M, Reichert TE. Salivary gland carcinomas. *Oral Maxillofac Surg.* 2012;16(3):267-83. PMID:22842859. <http://dx.doi.org/10.1007/s10006-012-0350-9>.
6. Simpson RHW, Skalova A, Di Palma S, Leivo I. Recent advances in the diagnostic pathology of salivary carcinomas. *Virchows Arch.* 2014;465(4):371-84. PMID:25172327. <http://dx.doi.org/10.1007/s00428-014-1639-x>.
7. Gupta R, Balasubramanian D, Clark JR. Salivary gland lesions: recent advances and evolving concepts. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2015;119(6):661-74. PMID:25840511. <http://dx.doi.org/10.1016/j.oooo.2015.02.481>.

Conflict of interest: None

Submitted on: May 11th, 2016

Accepted on: October 25th, 2016

Correspondence

Silvia Vanessa Lourenço

Dental School - Universidade de São Paulo

Av. Prof. Lineu Prestes, 2227 – São Paulo/SP – Brazil

CEP: 05508-000

Phone: +55 (11) 3091-7884

silvialourenco@usp.br