

## CASE REPORT

# Imprint cytological feature of large cell neuroendocrine carcinoma of the gallbladder : A case report

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**Abstract :** Large cell neuroendocrine carcinoma (LCNEC) is a rare poorly differentiated carcinoma with neuroendocrine differentiation showing aggressive clinical behavior. We herein report a case of gallbladder LCNEC, which was difficult to differentiate from poorly differentiated adenocarcinoma. An imprint cytology was very useful for the final diagnosis in this case. A 56-year-old male with left exophthalmos was admitted to the hospital. Radiological examinations revealed the presence of a left gallbladder tumor with orbital metastasis. The histological diagnosis was poorly differentiated adenocarcinoma, and intensive chemoradiotherapy was administered. Unfortunately, the patient died of extensive metastases 36 months after the initial onset of symptoms. An autopsy revealed a tumor mass in the gallbladder associated with multiple liver and peritoneal metastases. Imprint cytology of the main tumor revealed cytological features of LCNEC, and additional histological examinations confirmed this diagnosis. Although performing a histological examination is important for making a final diagnosis, imprint cytology is powerful tool for differential diagnosis of LCNEC, especially in patients with carcinoma with poor differentiation. *J. Med. Invest.* 60 : 149-153, February, 2013

**Keywords :** large cell neuroendocrine carcinoma, gallbladder, imprint cytology, screening

## INTRODUCTION

Large cell neuroendocrine carcinoma (LCNEC) is a high grade neuroendocrine tumors (NETs) with distinctive cytological and histological features. The current WHO classification categorizes NETs as grade 1, grade 2 and grade 3, with LCNEC and small cell carcinoma being categorized as grade 3 (1). LCNEC was originally reported to be a poorly

differentiated carcinoma with neuroendocrine differentiation arising from bronchopulmonary units showing aggressive clinical behavior (2). Extrapulmonary LCNEC has been established to be a rare malignant tumor associated with a poor prognosis. Gallbladder LCNEC accounts for 0.5% of all NETs and 2.1% of all gallbladder cancers (3). To date, although several case reports of gallbladder LCNEC have been published (4-7), reports with detailed cytological descriptions are rare. Since some cases of LCNEC show scarce histological features of neuroendocrine differentiation such as rosette-like structures and ribbon-like arrangements, making a histological diagnosis based on simple hematoxylin-eosin (HE) staining is occasionally difficult. We

Received for publication November 8, 2012 ; accepted December 7, 2012.

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herein report an autopsy case of gallbladder LCNEC in which a diagnosis was made using imprint cytology and additional immunohistochemistry. In this case, the histological diagnosis made based on the biopsy specimen was poorly differentiated adenocarcinoma. We discuss the advantages of imprint cytology in the detection of neuroendocrine differentiation of poorly differentiated carcinomas.

## CASE REPORT

A 56-year-old male was admitted to the hospital complaining of left exophthalmos. Radiographic examinations disclosed irregular thickening of the gallbladder wall with cholelithiasis, a left orbital tumor and multiple liver masses with enlarged abdominal lymph nodes. A biopsy sample of the surgically resected left orbital tumor showed that the atypical tumor cells proliferate with solid nest showing a few tubular formation, and was diagnosed for poorly differentiated adenocarcinoma (Figure 1). Although the patient underwent intensive chemoradiotherapy, systemic tumor metastasis developed and he died 36 months after the initial onset of symptoms. An autopsy revealed the presence of a large tumor in the hilum of the liver. The gallbladder was not distinguishable from the tumor mass due to extensive invasion. The tumor was whitish in color, measured 9×6×5 cm in size and showed direct invasion to the adjacent liver and duodenum. Additionally, multiple

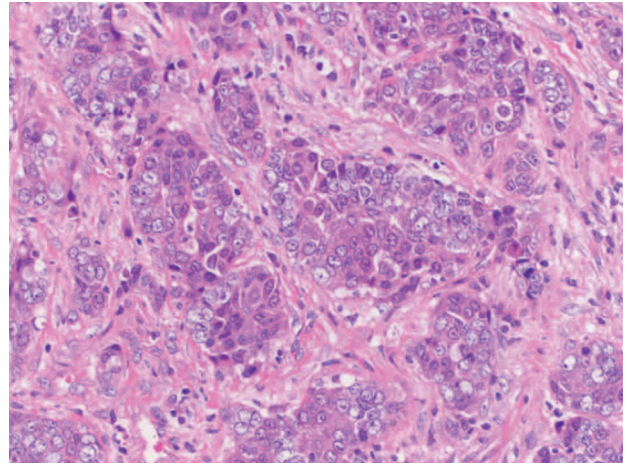


Figure 1. Histological findings of the surgically resected left orbital tumor. The formalin-fixed, paraffin-embedded tumor tissue was prepared with HE stain. The details of the findings are described in the text (Original magnification, ×200).

metastases in the liver, ileum, kidneys, adrenal glands, diaphragm, epicardium, left orbit and abdominal lymph nodes were observed.

The primary gallbladder tumor was subjected to imprint cytology and a conventional histological examination. For the cytological examination, imprinted cells placed on a slide were fixed with 95% ethanol and stained with Papanicolaou (Pap). The tumor cells of the gallbladder were clustered and arranged in a sheet-like pattern associated with a nuclear streaking or necrotic debris in the background (Figure 2a). The feature of tumor cells was

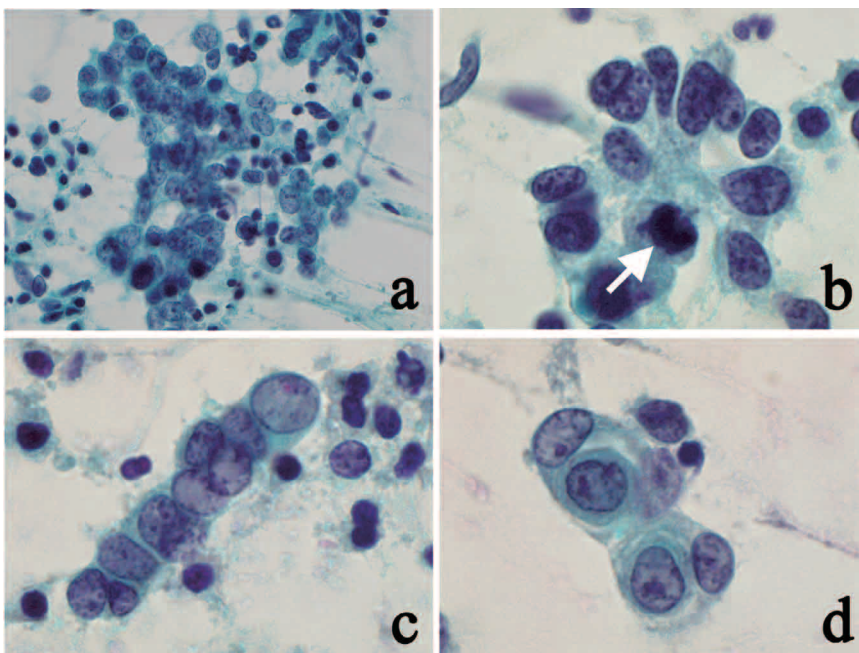


Figure 2. Cytological features of gallbladder LCNEC. The tumor cells were collected by imprinting to a slide and stained with Pap, as described in the Case report section. The details of the findings are described in the text (Original magnification, (a), ×400, (b), (c), (d), ×1,000).



round to oval in shape with an indistinct cellular border, and tumor cells had the palely light green-stained plentiful cytoplasm. The nuclei consisted of a thin nuclear membrane with fine or coarse granular chromatin and a few prominent nucleoli, and nuclei were larger than 3 to 4 times the diameter of a mature lymphocyte (Figure 2b-d). Although nuclear palisading was not evident in the periphery of the clusters, some of the tumor cells showed a rosette-like arrangement (Figure 2b), indian-filing (Figure 2c), cell cannibalism (Figure 2d) and mitotic figures (Figure 2b, arrow). Immunocytochemistry revealed that every tumor cell of a primary gallbladder tumor expressed CD56 (Figure 3). These cytological characteristics and immunocytochemical findings indicated a diagnosis of LCNEC (7-10).

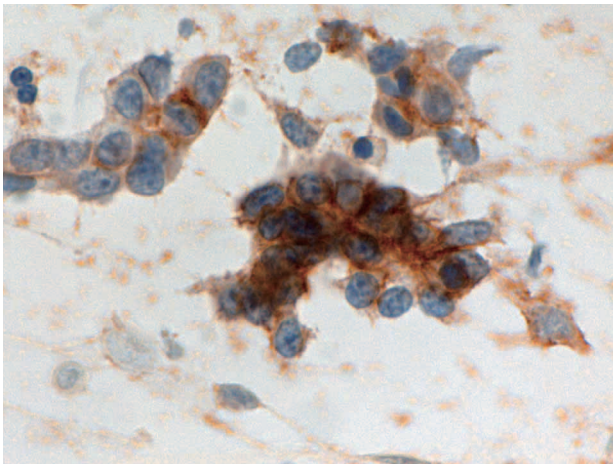


Figure 3. Immunocytochemical examination of gallbladder LCNEC. The tumor cells obtained from the primary tumor were immunostained, as described in the Case report section. The majority of tumor cells expressed neuroendocrine marker CD56 (Original magnification,  $\times 1,000$ ).

The tissue samples were fixed with 20% formalin and prepared as HE-stained sections. Histologically, the tumor was composed of numerous large tumor cells that showed desmoplastic reactions, and many areas of focal necrosis were seen in the tissue (data not shown). The tumor cells had polygonal cytoplasm and fine granular chromatin with prominent nucleoli (Figure 4) arranged in organoid or trabecular patterns; very few tumor cells showed rosette-like structures (Figure 4, inset). The mitotic figures were somewhat observed (2 mitoses per 10 high power fields). To identify the presence of neuroendocrine differentiation in the tumor cells, immunohistochemistry using the indirect immunoperoxidase method was performed. Table 1 summarizes

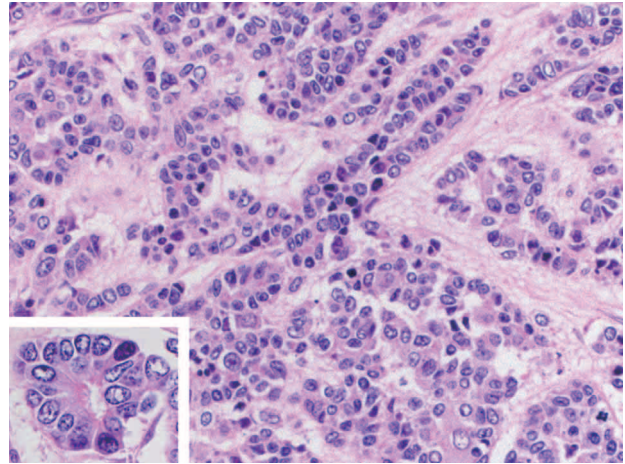


Figure 4. Histological evaluation of gallbladder LCNEC. The primary hepatic hiatal tumor was prepared as an HE stained tissue sample. The details of the findings are described in the text (Original magnification,  $\times 200$ ; inset  $\times 400$ ).

the primary antibodies used in this study. The immunostaining procedures were in accordance with the manufacturer's instructions (Histofine Simple Stain MAX-PO, Nichirei Bioscience, Tokyo, Japan). Immunohistochemistry revealed that every tumor cell of a primary gallbladder tumor expressed Chromogranin A (CGA), Synaptophysin (SYP) and CD56 (Figure 5), and they were negative for carcinoembryonic antigen (CEA). A retrospective immunohistochemical analysis of the biopsy sample from orbital tumor also showed the same result. The Ki-67 labeling index was 21%. The histological and immunohistochemical findings described above confirmed the final diagnosis of gallbladder LCNEC.

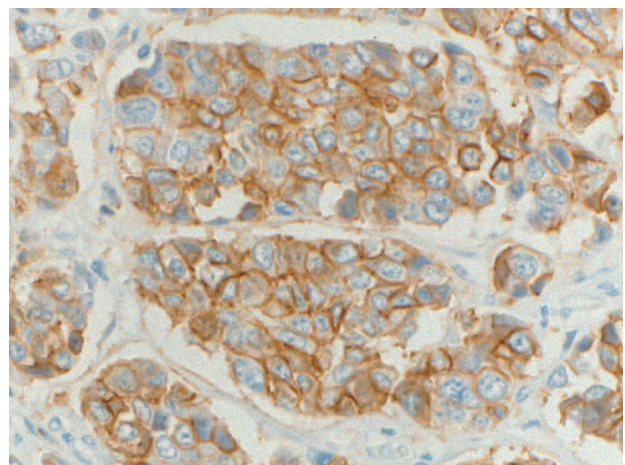


Figure 5. Immunohistochemical examination of gallbladder LCNEC. The tissue samples obtained from the primary tumor were prepared in paraffin blocks and immunostained, as described in the Case report section. The majority of tumor cells expressed neuroendocrine marker CD56 (Original magnification,  $\times 400$ ).

**Table 1.** List of primary antibodies and methods of antigen retrieval

Antibody	Manufacturer	Code number	Clone	Species	Dilution	Pretreatment
CGA	DAKO, Carpinteria, CA	A0430		Rabbit	× 500	MW (CB, pH 6)
SYP	DAKO	A0010		Rabbit	× 100	MW (CB, pH 6)
CD56	DAKO	M7304	123C3	Mouse	× 100	PCP (EDTA, pH 8)
CEA	DAKO	M7072	II-2	Mouse	× 50	MW (TRS, pH 6)
Ki-67	DAKO	M7240	MIB-1	Mouse	× 50	PCP (EDTA, pH 8)

Abbreviations : MW : microwave ; PCP : pressure chamber Pascal (DAKO) ; CB : citrate buffer ; TRS : target retrieval solution (DAKO)

## DISCUSSION

LCNEC arising from the hepatobiliary system is very rare and its prognosis is poorer than that of ordinary hepatobiliary carcinoma (4). To date, approximately 10 gallbladder LCNEC case reports have been published in the English literature (4-7). The latest report described the cytological features of the tumor cells in detail (7). Consistent with previous reports, the tumor cells in our case also demonstrated similar cytological features to those seen in bronchopulmonary LCNEC (7-10). The tumor cells in our case exhibited cell cannibalism ; this is not specific to LCNEC and is known to be associated with aggressive clinical behavior (11-13). In addition, this case involved multiple metastases, including an unusual orbital metastasis, which is a poor prognostic factor, especially in patients with gallbladder carcinoma (14, 15).

In this case, a histological examination using a biopsy of a surgically-resected tissue sample provided a diagnosis of poorly differentiated adenocarcinoma, however, a retrospective assessment revealed that very few tumor cells in the first biopsy sample and the autopsy samples showed rosette-like structures. Since the area of these structures was restricted, this finding was overlooked on the histological examination. In contrast, the nuclear features of the imprinted tumor cells shown in Figure 2b-d suggested a diagnosis of LCNEC, and rosette-like structures and Indian-filing were seen relatively often in the cytological specimens (Figure 2b, c). Another drawback of histology is the relatively indistinct nuclear pattern. Conventional HE-staining requires paraffin-embedding tissue preparation, which involves processed dehydration and deparaffinization. These procedures destroy and mask the details of nuclear structure. In contrast, cytology based on Pap stains does not require paraffin embedding, ensuring that the nuclear fine structure is well preserved. Therefore, cytology has an advantage in

making a qualitative diagnosis of LCNEC compared to HE-stained histological approaches. A previous report also describes the advantages of cytological examinations using endoscopic ultrasound-guided fine-needle aspiration samples in making early diagnoses of LCNEC in the biliary tract (7). Taken together, previous reports and the present case report indicate that imprint cytology is valuable for diagnosing LCNEC, especially in qualitative screening.

In summary, we reported the cytological features of a case of gallbladder LCNEC. Cytological screening of LCNEC is more accurate than histology because detailed nuclear findings provide valuable diagnostic information. In cases of poorly differentiated adenocarcinoma with an aggressive clinical course, we suggest adding aspiration cytology to conventional HE-stained histology of biopsy material. Further case studies are necessary to establish the value of cytology for LCNEC diagnosis.

## CONFLICT OF INTEREST

There is no conflict of interest to disclose.

## ACKNOWLEDGEMENTS

We thank Ms. Yui Hayashida and Ms. Emi Kiyota for their skillful technical assistance and Ms. Junko Hirai for her valuable secretarial efforts.

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