Mixed neuroendocrine-non-neuroendocrine neoplasm of the gallbladder: case report and literature review

Xu Ren1†, Hong Jiang2†, Kan Sun3, Xufu Qin4, Yongping Qu5, Tian Xia1 and Yan Chen6

Abstract
Background: Mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs) of the gallbladder are rare malignancies. Here we presented two cases and reviewed the related literature.

Case presentation: Our two patients were postoperatively diagnosed with gallbladder MiNENs, which pathologically consisted of a large cell neuroendocrine carcinoma and papillary adenocarcinoma. After cholecystectomy, one patient had a survival time of 30 months, while the other remained alive through 12 months of follow-up. In the literature, a total of 72 cases of gallbladder MiNENs were identified, and with our two patients included, we calculated a male-to-female ratio of 0.22 and a mean age of 64.5 years for the 74 reported cases. About one-half of these patients were found to have gallstones and presented with abdominal pain or discomfort in a relatively early stage. The preoperative diagnosis of these 74 cases mainly relied on abdominal ultrasound, contrast-enhanced computed tomography (CT) scanning, and magnetic resonance imaging or positron emission tomography/CT. However, the final diagnosis was established based upon the pathological evidence and expression of synaptophysin (Syn) and/or chromogranin A identified by immunohistochemical staining or neurosecretory granules detected by electron microscopy. Fifty-eight patients (78.4%) underwent various operations including simple cholecystectomy (n = 14), en bloc cholecystectomy (n = 9), standard or non-standard radical cholecystectomy (n = 25), or extended radical cholecystectomy (n = 6). The mean size of the resected gallbladder masses was 50.8 ± 36.1 mm (n = 63) with regional lymph node metastasis in 37 patients (52.1%), liver invasion or staging greater than T3 in 33 patients (45.8%), and hepatic metastasis in 26 patients (35.1%). The postoperative median survival time was 36 ± 11.42 months (95% confidence interval, 13.62 to 58.38 months). The log-rank analysis did not find that postoperative adjuvant chemotherapy contributed to a longer survival time relative to that among the patients who did not receive chemotherapy (numbers of patients, 15 versus 43; survival times, 36 months versus 30 months, p > 0.05).

Conclusions: Our two cases and the cases in the literature suggest that MiNENs of the gallbladder predominantly occur in women; are associated with early lymph node metastasis, local hepatic invasion, and hepatic metastasis; and can be managed by various surgeries as well as chemotherapy combined with somatostatin analogs.
Background

As an extremely rare pathological entity, mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs) pose inherent diagnostic and management challenges [1]. Based on the statistical results from Europe, the incidence of MiNENs is less than 0.01/100,000 cases per annum, and the common sites of origin of MiNENs are, in descending order, the appendix (60.3%), colon-rectum (14.5%), and rarely biliary tract (1.6%) [2], and two-thirds of cases in the biliary tract primarily arise from the gallbladder [3].

Neuroendocrine carcinomas (NECs) of the gallbladder only account for 4% of all malignant gallbladder neoplasms, and more than one-third of diagnosed gallbladder NECs coexist with an adenocarcinoma component (MiNENs) [1]. Clinically, MiNENs of the gallbladder that present as either cholelithiasis or gallbladder neoplasms have an insidious onset, are difficult to diagnose early, show rapid progression, and are associated with short survival time. Pathologically, MiNENs of the gallbladder generally are epithelial neoplasms but possess mixed pathophysiological natures of both a neuroendocrine neoplasm and adenocarcinoma, which are found to be more highly aggressive than gallbladder NEC alone in terms of regional lymph node and hepatic metastases [4]. This is partly attributed to the delay in their diagnosis and treatment [3], resulting in enhanced malignancy and a diminished long-term prognosis.

Our understanding of gallbladder MiNENs has been restricted by the rarity of this neoplasm and the limited amount of published data. Therefore, we reviewed the literature along with our case presentation to provide more information for improving the understanding of this disease to achieve early diagnosis and treatment.

Case presentation

Case one

A 70-year-old female patient with right upper abdominal pain for 4 days was admitted to our hospital on September 15, 2013, with gallstones and suspected gallbladder cancer. Despite a normal CA19–9 and neuron-specific enolase (NSE) level, the carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) levels were elevated at 8.04 ng/ml (normal: < 4.0 ng/ml) and 55.2 ng/ml (normal: 0.89 to 8.78 ng/ml), respectively. Abdominal ultrasound showed a 6-cm sized mass with an irregular and heterogeneous echogenicity (Fig. 1a) and a stone in the gallbladder. Contrast-enhanced computed tomography (CT) scanning and magnetic resonance imaging (MRI) further identified an enhanced gallbladder mass. Subsequently, open cholecystectomy was performed without major adverse events, during which hepatic metastases were not observed, but multiple enlarged lymph nodes in the hepatoduodenal ligament were found to infiltrate the portal vein and could not be dissected. Two years later, the patient was re-admitted on December 7, 2015, for...
obstructive jaundice and hepatic metastasis. The patient subsequently underwent endoscopic retrograde cholangiopancreatography (ERCP), in which a stricture in the middle extrahepatic bile duct (Fig. 1b) was identified and further managed with biliary stenting. Three months later, the patient died from systemic organ failure, with a survival time of 30 months. A lesson from this case is that any large size gallbladder lesions should be further investigated considering the possibility of MiNENs.

The gross findings of the incised gallbladder, in this case, showed a 70 mm × 50 mm soft polypoid mass in the neck and body, and a 2-cm stone in the gallbladder. Histopathological examination showed about 65% large cell neuroendocrine carcinoma (LCNEC) and 35% moderately differentiated papillary adenocarcinoma in the pathological sections with a distinct transitional zone between the two components (Fig. 2a). Large cells with a high mitotic rate (60 mitoses/2 mm²) were found in solid sheets or organoid nests, and also other microscopic characteristics of LCNEC were observed (Fig. 2b). Additionally, cancer emboli were observed in the lymphatic vessels. Meanwhile, LCNEC invaded the gallbladder, while papillary adenocarcinoma invaded the subserosal layer. No metaplastic mucosa was seen around the tumor. On December 7, 2015, the pathology of the bile duct biopsy from ERCP after recurrence identified only well-differentiated papillary adenocarcinoma and not the LCNEC component (Fig. 2c). The immunohistochemical staining results for MiNENs of the gallbladder in the cases are shown in Table 1.

**Case two**

A 64-year-old female patient presented to our hospital on May 2, 2020, with a 1-week history of epigastric pain, nausea, and vomiting. Physical examination was only notable for localized abdominal tenderness. Preoperatively, all laboratory tests, including levels of tumor markers CEA, CA19–9, and NSE were normal. Ultrasonography revealed a wide-base nodular projection in the gallbladder, and further imaging studies including magnetic resonance cholangiopancreatography (MRCP), CT, and ¹⁸F-fluorodeoxyglucose (¹⁸FDG)-positron emission tomography (¹⁸FDG-PET)/CT demonstrated a hypointense mass with a scattered, mildly calcified shadow in the gallbladder (Fig. 3a) and abnormal FDG accumulation in the mass (Fig. 3b), respectively, all of which suggested gallbladder cancer. Thus, the patient underwent en bloc cholecystectomy with hepatoduodenal ligament lymph node dissection.

In this case, a hard semipedunculated nodule with the size of 25 mm × 25 mm (Fig. 4a) was observed in

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**Fig. 2** Case 1: Histological findings of MiNEN in the gallbladder by hematoxylin and eosin (HE) staining. a Two components, LCNEC (right) and papillary adenocarcinoma (left) showed mixed composition and solid sheet distribution, with an obvious transitional zone between the two tissues. Magnification, × 100. b Large cells arranged in solid sheets, with vesicular nuclei and abundant eosinophilic cytoplasm, and tumor cells had large-sized densely stained round to oval nuclei, in some cells visible nucleoli, high mitotic index (arrows), and focal tumoral necrosis consistent with LCNEC were showed. Magnification, × 400. c Metastatic lesion of the bile duct showing well-differentiated papillary adenocarcinoma. Magnification, × 100
the body of the gallbladder without gallstones. This neoplasm contained two cellular components with a composition ratio matching that of case 1 and was more significantly distributed in an organoid nest with a mixed transitional zone (Fig. 4b). A high mitotic rate (35 mitoses/2mm²) was observed, and the microscopic findings for LCNEC are shown in Fig. 4c. Moreover, LCNEC invaded the subserosal layer, and cancer emboli were observed in both blood vessels and lymphatic vessels. Metastasis in the regional lymph nodes was found predominantly with LCNEC components. The immunohistochemical staining results for MiNENs of the gallbladder, in this case, are shown in Table 1.

Table 1 Immunohistochemical staining findings in two cases of gallbladder MiNENs

| Antibody | Case 1 | | Case 2 |
|----------|--------|--------|--------|
|          | LCNEC  | PAC    | LCNEC  | PAC    |
| Syn      | diffusely strong positive | – | diffusely strong positive | – |
| CgA      | weakly positive | – | diffusely strong positive | – |
| CEA      | – | positive | – | positive |
| AE1/AE3  | spotted weakly positive | diffusely strong positive | diffusely strong positive | diffusely strong positive |
| LCA      | – | – | – | – |
| CD117    | – | – | – | – |
| CD34     | – | – | – | – |
| CK20     | – | – | – | – |
| CK7      | – | diffusely positive | – | diffusely positive |
| CDX2     | – | – | – | – |
| PS3      | overexpression | overexpression | – | – |
| Ki67     | 80% | 40% | 80% | 60% |

PAC Papillary adenocarcinoma, Syn Synaptophysin, CgA Chromogranin A, overexpression: > 80%; –: Null

![Fig. 3](image-url) Case 2: 18FDG-PET/CT examination. a An indistinct hypointense mass and scattered slightly hyperdense calcified shadow in the gallbladder were observed. b FDG accumulated in the gallbladder mass

The immunohistochemical staining for synaptophysin (Syn), chromogranin A (CgA), AE1/AE3, tumor protein 53 (TP53), and Ki67 in both cases is shown in Figs. 5 and 6, respectively.

Literature review
We found 72 case reports of gallbladder MiNENs in the literature, and along with the two cases presented above, proceeded with this review (Table 2). In our statistical analyses, the categorical variables were expressed as numbers and percentages, and the continuous variables were expressed as medians and ranges. Median survival outcomes were estimated by applying Kaplan–Meier analysis; moreover, the log-rank test was used to evaluate differences between groups. All data were analyzed using SAS9.4 statistical software.

Demographically, the 74 patients had a mean age of 64.5 years, ranging from 36 to 85 years, with a ratio of male to female patients of 0.22. Clinically, more than two-thirds of patients presented with right upper quadrant or epigastric pain or discomfort (n = 34, 68%), and just over one-half were found to also have gallstones.
A few patients developed obstructive jaundice and weight loss, but some were asymptomatic. Preoperatively, enhanced CT and MR images showed enhancement of a homogeneous irregular mass as a high-intensity tumor, and $^{18}$FDG-PET/CT could detect accumulation of $^{18}$FDG in a mass or thickened gallbladder wall for poorly differentiated NECs. For cases in which difficulty occurred in establishing the diagnosis, ultrasound or CT- and endoscopic ultrasonography (EUS)-guided biopsy had diagnostic value. Tumor marker expression was not checked for all patients preoperatively, and among the 74 patients, the CEA level was only examined in 14 patients, of which five (35.7%) had an elevated CEA level in the range of 8 to 43 ng/ml (mean 22.6 ng/ml, normal < 5 ng/ml). The CA19–9 concentration was elevated in 11 of 20 patients (55.0%), ranging from 73 to 728 U/ml (mean 215.3 U/ml, normal < 37 U/ml). An increase in AFP was found in 2 of 6 cases for which AFP was included in the work-up (157,428 ng/ml and 55.2 ng/ml).

Therapeutically, of the 74 patients with gallbladder MiNENs, 58 were treated surgically (78.4%), including 14 cases treated by simple cholecystectomy, 9 cases treated by cholecystectomy with gallbladder fossa liver tissue or liver bed wedge resection, 7 cases treated by cholecystectomy plus hepatectomy, 3 cases treated by cholecystectomy with regional lymph node dissection, 15 cases treated by en bloc cholecystectomy with hilar lymph node dissection (Glenn operation) or hepatectomy with hilar lymph node dissection, 6 cases treated by extended radical cholecystectomy (ERC) resection or extended to hepatopancreaticoduodenectomy, and 4 cases treated by palliative operations (Table 2).

Among the neuroendocrine components of MiNENs in the gallbladder, NEC without specified pathological subclassification (NSNEC) was the most common ($n=28$, 37.8%), followed by small cell neuroendocrine carcinomas (SCNEC, $n=24$, 32.4%), LCNEC ($n=18$, 24.3%), and neuroendocrine tumours (NET) ($n=4$, 5.4%). The non-neuroendocrine component, predominantly, was adenocarcinoma only, but in 14.9% of patients ($n=11$), two or more non-neuroendocrine components co-existed ($n=9$), mainly adenocarcinoma with squamous cell carcinoma (Table 2), or two synchronous neuroendocrine components were present ($n=2$). The vast majority ($n=64$, 92.8%) had a mass with a nodular, giant, or polypoid pattern. The mass sizes were reported in 63 case reports and ranged from 10 to 150 mm (mean, 50.8 ± 36.1 mm). Most masses showed a sessile (type 1s) or semipedunculated (type 1p or 1s) appearance.
Isp) morphology, and a few (4.8%) were pedunculated (type Ip). A small number of patients were found to have non-mass MiNENs ($n = 5, 7.3\%$), including 4 cases of localized or diffuse thickening of the gallbladder wall and one case of the multilocular cystic tumor (MCN).

The histological features of vascular invasion have been documented in the literature. For lymphovascular infiltration, in a case series with 13 cases, four patients had lymphatic invasion, while in another 15 cases reports, 10 cases had vascular invasion, of which only one had liver metastasis. Because the number of patients was low and there was no endpoint time in the group without lymphatic or vascular invasion, the median survival time could not be calculated.

Moreover, nearly half of the patients had liver invasion with staging above T3 ($n = 33, 45.8\%$), and more than half had regional lymph node metastasis ($n = 37, 52.1\%$). One-third had liver metastasis ($n = 26, 35.1\%$), and a few had metastasis of the bone, lung, skin, other abdominal organs (adrenal gland, pancreas), or peritoneal metastasis. Occasionally MiNENs in the gallbladder metastasized to the eyeball or femoral head. The median survival time of MiNEN patients ($n = 59$) was $36 \pm 11.42$ months (95% confidence interval (CI) 13.62 to 58.38 months; Fig. 7). Approximately one-fourth of cases received postoperative adjuvant chemotherapy (PAC) ($n = 15, 25.9\%$) with a median survival time of $36 \pm 15.46$ months (95% CI, 5.70 to 66.30 months). In comparison, the median survival time of 43 patients who did not receive PAC was 30 months. Log-rank analysis was used to compare the survival times of patients who did or did not receive postoperative adjuvant chemotherapy, and the log-rank

![Fig. 5 Case 1: Immunohistochemical staining findings for LCNEC and adenocarcinoma. a Syn staining was diffuse and strongly positive in LCNEC and negative in adenocarcinoma. Magnification, × 40. b Staining for CgA was weakly positive in LCNEC and negative in adenocarcinoma area. Magnification, × 40. c High Ki67 proliferation index was found in LCNEC and the adenocarcinoma component. Magnification, × 40. d AE1/AE3 staining was strongly positive in adenocarcinoma, and punctate weak positive staining was observed in LCNEC. Magnification, × 40. e TP53 staining showed overexpression in the LCNEC component (left) and in the adenocarcinoma component (upper right). Magnification, × 40. f TP53 staining also showed overexpression in the adenocarcinoma component (> 80%). Magnification, × 40.](image-url)
comparison statistic was 0.15 ($P=0.698$), indicating the difference was not statistically significant.

**Discussion and conclusions**

MiNEN of the gallbladder is an extremely rare disease that is more common in women than men with a male-to-female ratio of 0.22. The majority of patients with MiNENs presented with abdominal pain or discomfort or merely cholelithiasis-like symptoms in the early stage, and did not develop any symptom of carcinoid syndrome as the initial presentation, indicating carcinoid syndrome-causing chemicals might not be produced, or just sequestered in the biliary system in the early phase of the disease. Most gallbladder NiNENs formed a nodular or polypoid mass, which could develop into a large mass that invades adjacent organs such as the liver. A few cases were characterized by features of either localized or diffuse gallbladder wall thickening or degeneration due to tumor necrosis.

Histopathologically, the gallbladder MiNEN contains two tumor components of neuroendocrine and non-neuroendocrine type, with $\geq 30\%$ of each component. Usually, the neuroendocrine component coexists with adenocarcinoma, but rarely is it found with other rare cancers or with two or more non-neuroendocrine components, such as squamous cell carcinoma, adenosquamous carcinoma, undifferentiated carcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, sarcomatoid, osteosarcomatous and intracholecystic papillary neoplasm (ICPN), etc. [27, 51]. Also, two neuroendocrine components such as LCNEC and SCNEC can coexist with one adenocarcinoma [28]. The origin of MiNENs remains unclear since the normal gallbladder mucosa does not contain neuroendocrine cells, except for the gallbladder neck region [43]. Immunohistochemistry of gallbladder MiNENs in our case simultaneously revealed $TP53$ overexpression and a high Ki67 proliferation index in LCNEC and adenocarcinoma of two different epithelial tumors, thereby suggesting that the two different components had the same molecular background [3, 51].

Moreover, the gallbladder NECs has two subclasses as SCNEC and LCNEC [4]. In this review of 74 cases of MiNENs of the gallbladder, NEC without specified pathology was the most common type (37.8%), followed by SCNEC (32.4%), then LCNEC (24.3%), and NETs (5.4%). The reason for the terminology of NEC rather that either SCNEC or LCNEC being more commonly used in many case reports might result from the adoption of the previous World Health Organization (WHO) classification of NENs of the digestive system. Furthermore, the current review found that patients with MiNENs of the gallbladder most likely had higher regional lymph node metastasis (50.7%) and hepatic metastasis.
| Case No. | Author [ref.] | Year | Age (yr)/ sex | Tumor size (mm) | Invasion depth | Metastasis Lymph node | Liver | Ki-67(%) / mitoses (/2mm²) | NEN component | no-NEN component | Surgery and/or chemotherapy | Outcome (months) |
|---------|---------------|------|---------------|-----------------|----------------|-----------------------|-------|--------------------------|----------------|------------------|-----------------------------|-----------------|
| 1       | Wisniewski et al. [5, 6] | 1972 | 51/F | Large | hinf | – | + | NM | NEC | AC | Cholecystectomy with L | Death (NM) |
| 2       | Ito et al. [5, 6] | 1980 | 75/F | 60 × 35 × 20 | NM | – | – | NM | NEC | Undifferentiated carcinoma | Cholecystectomy | Death (13) |
| 3       | Wada et al. [7] | 1983 | 56/M | 55 × 40 × 28 | ss | – | – | NM/rarely | NET | Papillary AC | Cholecystectomy with RL | Death (16) |
| 4       | Muto et al. [8] | 1984 | 80/M | 10 × 7 × 7 | ss | – | – | NM | NET, goblet cell adenocarcinoid | AC | Cholecystectomy with hepatic bed resection and RL | Survival (24) |
| 5       | Masuo et al. [5] | 1984 | 74/M | NM | NM | – | + | NM | NEC | AC | Cholecystectomy | Autopsy | Death (3) |
| 6       | Kotake et al. [5, 6] | 1984 | 47/F | 15 × 10 | ss | + | + | NM | NEC | AC | Cholecystectomy | Death (17) |
| 7       | Haga et al. [5] | 1988 | 79/M | 35 | hinf | – | – | NM | NEC | AC | Cholecystectomy with hepatic bed resection | Death (10) |
| 8       | Kurosaka et al. [5] | 1988 | 46/F | 40 | ss | + | + | NM | NEC | AC | Cholecystectomy with hepatic bed resection | Survival (4) |
| 9       | Adachi et al. [9] | 1988 | 69/F | 55 × 30 | hinf | – | – | NM | NEC | AC | Cholecystectomy with hepatic bed resection | Survival (12) |
| 10      | Yamamoto [6] | 1989 | 76/F | 25 × 25 | ss | – | – | NM/Common | NEC | Well-differentiated AC | Cholecystectomy | RF (9) |
| 11      | Fish et al. [10] | 1990 | 77/F | Small polyoid | ss | – | – | NM/many | NEC | AC | Cholecystectomy with hepatic bed resection | RF (7) |
| 12      | Ohn et al. [5] | 1991 | 63/F | 140 | ss | + | + | NM | NEC | AC | Cholecystectomy with hepatic bed resection | Death (9) |
| 13      | Cavazzana [11] | 1991 | 71/F | 50 × 30 × 30 | T3 | + | + | NM | SCNEC | Well-differentiated AC | Cholecystectomy with RL | Death (4) |
| 14      | Duan et al. [52] | 1991 | 70/M | 10 | ss | + | + | NM | SCNEC | AC | Autopsy | Death (1) |
| 15      | Lida [12] | 1992 | 62/F | 65 X 30 | Hinf | + | + | NM/frequently | SCNEC | AC, squamous cell carcinoma | Cholecystectomy with L | Death (5) |
| 16      | Ohmori et al. [13] | 1993 | 78/F | 40 × 30 | T3 | N2 | M1 | NM/frequently | NEC | AC | Autopsy | Death (1/4) |
| 17      | Murayama et al. [5] | 1997 | 68/F | 20 | se | + | + | NM | NEC | AC | Cholecystectomy | Death (2) |
| Case No. | Author [ref.] | Year | Age (yr)/ sex | Tumor size (mm) | Invasion depth | Metastasis | Ki-67(%) / mitoses (/2mm²) | NEN component | no-NEN component | Surgery and/or chemotherapy | Outcome (months) |
|----------|---------------|------|---------------|----------------|---------------|------------|----------------|-----------------|----------------|-------------------------------|------------------|
| 19       | Yokoyama et al. [5] | 1998 | 72/M | 25 | hinf | + + | NM | NEC | AC | Cholecystectomy with hepatic bed resection | Survival (7) |
| 20       | Kamisawa et al. [5] | 1998 | 48/F | 50 | hinf | + + | NM | NEC | AC | Autopsy | NM |
| 21       | Furukawa et al. [5] | 1998 | 68/M | 35 | hinf | – – | NM | NEC | AC | Cholecystectomy with hepatic bed resection | Survival (18) |
| 22       | Moskal et al. [14] | 1999 | 69/F | NM | T3 | N2 | M0 | NM | SCNEC | Poorly differentiated AC | Chemotherapy, ERC | Survival (44) |
| 23       | Moskal et al. [14] | 1999 | 40/M | NM | T2 | N1 | M0 | NM | SCNEC | Moderately differentiated AC | ERC, chemotherapy | Survival (189) |
| 24       | Moskal et al. [14] | 1999 | 71/F | NM | T2 | N2 | M1 | NM | SCNEC | Poorly differentiated AC | Palliative surgery, chemotherapy | Death (13) |
| 25       | Papotti et al. [15] | 2000 | 50/M | Thickened GB wall | ss | – – | 50/>20 | LCNEC | AC | Cholecystectomy | RF (12) |
| 26       | Sakaki et al. [16] | 2000 | 79/F | 33 x 20 x 16 | m | – – | NM | SCNEC | AC | Cholecystectomy | RF (8) |
| 27       | Inguchi et al. [5] | 2000 | 81/F | 26 x 16 | ss | – – | NM | NEC | Papillary AC, signet-ring cell carcinoma | Cholecystectomy | RF (8) |
| 28       | Yannakou [17] | 2001 | 72/F | 70 x 62 x 16 | hinf | + + | NM/high rate | NEC | Well-differentiated AC | Radical cholecystectomy | Death (2) |
| 29       | Maitra et al. [18] | 2001 | 85/F | 40 | mp | – + | NM | SCNEC | AC | NM | Survival (13) |
| 30       | Maitra et al. [18] | 2001 | 77/F | 28 | T3 | + + | NM | SCNEC | AC | NM | Survival (25) |
| 31       | Maitra et al. [18] | 2001 | 73/F | 25 | mp | + – | NM | SCNEC | AC | NM | Survival (7) |
| 32       | Maitra et al. [18] | 2001 | 82/M | 10 | mp | – + | NM | SCNEC | AC, squamous cell carcinoma | NM | Survival (8) |
| 33       | Piana et al. [19] | 2002 | 66/F | 18 | SS | – – | NM/higher | SCNEC | Clear cell AC | Cholecystectomy, chemotherapy | Death (36) |
| 34       | Wakahayashi [20] | 2003 | 71/F | 100 | Se | – – | NM | NEC | AC, squamous cell carcinoma | Chemotherapy, extended liver resection | Survival (36) |
| 35       | Okamoto et al. [21] | 2003 | 70/M | 37 x 22 | T3 | + + | NM | SCNEC | Papillary AC | Chemotherapy, chemotherapy with L | Survival (0.5) |
| 36       | Koe et al. [22] | 2004 | 68/F | MCN | se | + – | NM | NEC | AC | Palliative surgery, chemotherapy | Death (6) |
| Case No. | Author [ref.] | Year | Age (yr)/ sex | Tumor size (mm) | Invasion depth | Metastasis Lymph node Liver | Ki-67(%) / mitoses (/2mm²) | NEN component | no-NEN component | Surgery and/or chemotherapy | Outcome (months) |
|----------|---------------|------|---------------|----------------|----------------|-------------------------|---------------------------|---------------|----------------------|--------------------------|------------------|
| 37       | Mori et al. [20] | 2005 | 70/F          | 36 x 15        | +             | –                       | NM                         | NEC | AC, squamous cell carcinoma | Cholecystectomy with hepatic bed resection | Survival (32) |
| 38       | Shimizu et al. [23] | 2006 | 58/M          | 150 x 90 x 120 | hinf          | NM                       | –                         | NM | SCNEC | AC | Cholecystectomy with hepatic trisegmentectomy | Death (4) |
| 39       | Noske [24] | 2006 | 81/F          | 50 x 35 x 30   | T3            | N1 M1                   | NM                         | LCNEC | Adenosquamous carcinoma | Palliative surgery | NM |
| 40       | Tsuchiya et al. [25] | 2006 | 36/F          | 10 x 8         | ss            | –                       | –                         | NM | NEC | Papillary AC | ERC | RF (12) |
| 41       | Sośnic and Sośnic [26] | 2006 | 56/F          | Thickened GB wall | hinf          | –                       | –                         | NM | NEC | Papillary AC | Cholecystectomy with biliary-enteric anastomosis | Survival (0.3) |
| 42       | Hashimoto [27] | 2007 | 55/F          | 18 x 12 x 5    | ss            | –                       | –                         | NM | NEC | AC, mucinous AC | Radical cholecystectomy | Survival (1.8) |
| 43       | Oshiro et al. [28] | 2008 | 55/F          | 49 x 45        | ss            | –                       | –                         | 73.3/NM 62.5/NM | LCNEC | SCNEC | AC | ERC | RF (20) |
| 44       | Iype et al. [29] | 2009 | 85/M          | 14 x 15        | se            | NM                       | +                         | NM | LCNEC | AC | Cholecystectomy, chemotherapy | Death (21) |
| 45       | Taniguchi et al. [30] | 2009 | 62/M          | 100            | T4            | +                       | –                         | NM | SCNEC | AC | Chemotherapy, autopsy | Death (8) |
| 46       | Sato et al. [31] | 2010 | 68/F          | 35             | hinf          | +                       | –                         | 72/>50 | LCNEC | Well-differentiated AC | Cholecystectomy with L | RF (1.2) |
| 47       | Kim et al. [32] | 2011 | 48/F          | 95 x 93 x 65   | T3            | +                       | –                         | NM | SCNEC | Moderately differented AC | ERC and chemotherapy | RF (18) |
| 48       | Paniz Monodolfi [33] | 2011 | 48/F          | 35 x 33 x 24   | hinf          | +                       | +                         | NM/>20 | LCNEC | Papillary AC | Cholecystectomy with hepatic bed resection | NM |
| 49       | Harada et al. [34] | 2012 | 70/F          | 35 x 2.5       | hinf          | +                       | –                         | 12.3/59 | SCNEC | Well-differentiated AC | NM | NM |
| 50       | 2012 | 70/F          | 45 x 10       | se            | –                       | –                         | 32.3/137 | LCNEC | Well-differentiated papillary AC | NM | NM |
| 51       | 2012 | 70/F          | 45 x 25       | T3            | –                       | –                         | 0.5/4 | NET G2 | Well-differentiated AC | NM | NM |
| 52       | 2012 | 60/F          | 15 x 15       | ss            | +                       | –                         | 28.5/95 | SCNEC | Well-differentiated papillary AC | NM | NM |
| Case No. | Author [ref.] | Year | Age (yr)/ sex | Tumor size (mm) | Invasion depth | Metastasis | Ki-67(%) / mitoses (1/2mm²) | NEN component | no-NEN component | Surgery and/or chemotherapy | Outcome (months) |
|----------|---------------|------|----------------|-----------------|---------------|-------------|-----------------|----------------|----------------------|-----------------------------|-----------------|
| 53       |               | 2012 | 50/F           | 150 x 120       | hinf          | + –         | 15.1/42       | LCNEC          | Well-differentiated AC | NM                          | NM              |
| 54       | Song et al. [35] | 2012 | 55/F           | 7 0 x 30 x 20   | T3            | – –         | >80/> 20      | SCNEC          | Moderately differentiated AC | RF (7)              |
| 55       | Rastogi et al. [36] | 2012 | 48/F           | Thickened GB wall | T3            | – –         | NM             | NEC            | Cholecystectomy with hepatic bed resection and L | NM              |
| 56       | Fuji et al. [37]  | 2012 | 72/F           | 100             | T2b           | N2 M1       | 28/NM         | SCNEC          | AC                       | Death (2)              |
| 57       | Russo et al. [38] | 2012 | 59/F           | 45 x 40         | hinf          | + +         | NM/NM         | LCNEC          | Mucinous carcinoma       | Survival (24)          |
| 58       | Al-Brahim [39] | 2013 | 45/M           | 57 x 55 x 51   | T3            | + +         | > 95/50       | LCNEC          | AC                       | Survival (8)           |
| 59       | Shintaku [40]   | 2013 | 80/M           | 82 x 53 x 50   | In situ       | – –         | 18.7/6.2      | NET G2         | Well-differentiated AC, squamous cell carcinoma | | |
| 60       | Abe et al. [20] | 2013 | 81/F           | 20 x 40         | ss            | + –         | NM             | NEC            | AC, squamous cell carcinoma | Cholecystectomy with hepatic bed resection and RL, survival (48) |
| 61       | Chen et al. [41] | 2014 | 34/F           | 40              | T3            | + –         | > 50/NM       | NEC            | AC                       | Survival (4)           |
| 62       | Meguro [42]     | 2014 | 54/F           | 90 x 60         | T2            | – –         | 80/NM         | LCNEC          | Poorly differentiated AC (ICPN) | RF (2.4)            |
| 63       | Chatterjee et al. [43] | 2014 | 73/F           | 15 x 6 x 6     | m             | – –         | NM/60         | SCNEC          | Moderately differentiated papillary AC | Cholecystectomy with hepatic bed resection and RL, survival (45) |
| 64       | Liu et al. [44] | 2015 | 63/F           | 20              | T2a           | – –         | > 80/NM       | LCNEC          | AC                       | Radical cholecystectomy | RF (12)            |
| 65       | Acosta et al. [45] | 2015 | 55/F           | 35 x 24 x 12   | se            | + –         | NM/27         | LCNEC          | Well-differentiated AC | Cholecystectomy | NM              |
| 66       | Kamboj et al. [46] | 2015 | 65/F           | NM              | T3            | – +         | NM             | NEC            | AC                       | Biopsy                   | Survival (2) |
| 67       | Azad et al. [47] | 2015 | 62/F           | 20 x 20         | se            | – –         | 15/NM         | NEC            | Moderately differentiated AC | Radical cholecystectomy | RF (24) |
| Case No. | Author [ref.] | Year | Age (yr)/ sex | Tumor size (mm) | Invasion depth | Metastasis | Ki-67(%) / mitoses (/2mm²) | NEN component | no-NEN component | Surgery and/or chemotherapy | Outcome (months) |
|----------|--------------|------|---------------|----------------|---------------|------------|----------------|----------------|----------------|--------------------------------|-----------------|
| 68       | Jung et al. [48] | 2018 | 54/F | 43 × 40 | T3 | + | + | NM/33 | LCNEC | Adenosquamous carcinoma | Radical cholecystectomy, chemotherapy | Death (13) |
| 69       | Lin et al. [49] | 2018 | 43/F | 74 × 56 | T3 | – | – | 85/NM | SCNEC | Poorly differentiated AC | Radical cholecystectomy, chemotherapy | Survival (21) |
| 70       | Ines et al. [3] | 2019 | 74/F | 61 | se | – | – | 95/83 | LCNEC | Well-differentiated AC | Cholecystectomy | Survival (7) |
| 71       | Skalický et al. [50] | 2019 | 56/F | 150 | T4 | + | + | 70/64 | SCNEC | AC | Cholecystectomy with L, chemotherapy | Survival (13) |
| 72       | Sciarra et al. [51] | 2020 | 66/F | 95 | m | – | – | NM | LCNEC | AC, ICPN | Cholecystectomy with hepatic bed resection and RL | NM |
| 73       | Present | 2020 | 70/F | 70 × 50 | mp | + | – | 80/>60 | LCNEC | Well-differentiated papillary AC | Cholecystectomy | Death (30) |
| 74       | Present | 2020 | 64/F | 25 × 25 | ss | + | – | 80/>60 | LCNEC | Well-differentiated papillary AC | Cholecystectomy with hepatic bed resection and RL | Survival (1.2) |

NEN Neuroendocrine neoplasm, M Male, F Female, NM Not mentioned, NEC neuroendocrine carcinoma, AC Adenocarcinoma, NET Neuroendocrine tumor, RF recurrence-free, SCNEC Small cell neuroendocrine carcinoma, ERC Extended radical cholecystectomy, LCNEC Large cell neuroendocrine carcinoma, MCN Multilocular cystic neoplasm, GB Gallbladder, G2 Grade 2, ICPN Intracholecystic papillary neoplasm, m Mucosal layer, mp Muscle propria, ss Subserosal invasion: tumor penetrated the serosa without invasion of adjacent structures, hinf Hepatic infiltration; cholecystectomy with RL: cholecystectomy with the cleaning of the regional lymph nodes; cholecystectomy with L: cholecystectomy with segmental liver resection.
rates (34.3%) compared with 15 and 17% of NEC of the gallbladder, respectively [4], suggesting that two co-excit-
ing cancerous components may be one of the potential pathogenic mechanisms for enhanced invasiveness, even though some data reveal that the grade of the neuroen-
docrine component correlates with prognosis [23, 48]. Fifty-eight postoperative patients with gallbladder MiN-
ENs had a short survival time of 36 months, although it seemed longer than the overall survival (25 months) of 754 patients with gallbladder NEC [4]. This difference in survival time might be affected by differences in the thera-
pies applied.

Importantly, the size of NEC is not necessarily pro-
portional to the metastatic potential, with the evidence indicating that even small primary NEC lesions also may infiltrate deeply or develop distant metastasis. In our review, three of four gallbladder MiNENs with a tumor size of 1 cm developed local invasion beyond the subse-
rosal layer, and two (50%) led to liver metastasis and/or lymph node metastasis, one of which produced extensive metastasis to the liver, rectum, lung, adrenal gland, and pancreas before detection of the primary lesion of the gallbladder on ultrasound or CT examination [52]. In addition, of all seven T1 tumors in the review, except for one case of carcinoma in situ and two cases of mucosal cancer, three SCNEC cases and one LCNEC case with T1 stages were found to have lymph node or liver metastasis. Thus, MiNEN of the gallbladder may possess early meta-
static potential.

It has been noted that the two different histological types of MiNEN of the gallbladder often metastasize sepa-
rateley [48]. In other words, the synchronous metastatic hepatic nodule is only composed of one component of MiNEN, while the metachronous metastatic hepatic nodules may be composed entirely of another component. However, this usually depends on the metastatic potential of each pathological component in the MiNEN [24, 48]. In our first case, the tumor metastasis and infiltration to the middle extrahepatic bile duct was found to be papillary adenocarcinoma without the LCNEC component. The sur-
vival of MiNEN patients mainly depends on NEC, which is closely related to lymph node and liver metastasis [25]; however, in case one, metastasis of the adenocarcinoma may have been the cause of death for this patient. There-
fore, the malignancy of the two components of MiNENs may be separate determinants of the long-term prognosis.

Technically, the neuroendocrine components of MiNEN of the gallbladder can be determined by immu-
nolabeling. Strong positive staining for Syn, CgA, NSE, somatostatin, etc. [46], and ultrastructural electron microscopy, even if a small number of neurosecretory granules (NSG) are found, all help the identification of neuroendocrine cells to establish the diagnosis of NEN [23]. Duan et al. [52] reported cases of coexistence of SCNEC and adenocarcinoma of the gallbladder, and the small cell carcinoma was noted with only weakly posi-
tive immunoexpression for NSE and a negative reaction to argentaffin staining or staining for other neurose-
cretory markers including CgA. However, electron micro-
scopic examination, on the other hand, revealed NSG in the cytoplasm of some tumor cells, suggesting neuroen-
docrine tumors. Detection of serum NSE and CgA levels can also be used for diagnosis. The present two cases of MiNENs of the gallbladder showed positive expression
of both Syn and CgA on immunohistochemical staining of LCNEC, and further histological analysis showed large and pleomorphic cells arranged in solid sheets or organoid nests. Moreover, the tumor cells had large-sized round to oval nuclei with visible vesicular nuclei, some visible prominent nucleoli, abundant cytoplasm, quick mitotic activity (exceeding 20 mitoses/2 mm²), a high Ki67 index of over 20%, and frequent large areas of necrosis, features consistent with the characteristics of LCNEC [4, 39, 44, 53].

Clinically, the diagnosis of MiNEN mainly relies on various imaging studies. Abdominal ultrasonography as a first imaging modality showed hypoechoic irregular solid nodules of heterogeneous internal echoes with increased blood flow that may be characteristic of NEC of the gallbladder. On contrast-enhanced CT scanning and MRI examination of the gallbladder, MiNENs appeared as an irregular mass, homogeneous, and strongly enhanced as high-intensity tumors [20]; however, the enhanced tumorous lesions could not be differentiated from gallbladder cancer [28]. In patients with localized thickening of the gallbladder wall, CT scanning without enhancement revealed a low-intensity thickening of the gallbladder wall or the appearance of debris [25], and CT with contrast showed heterogeneous ill-defined soft tissue enhancement along the gallbladder fossa. MRI showed local non-enhanced areas of altered signal intensity, indicating the presence of cystic degeneration [36]. The diagnosis has been difficult to establish for MiNENs of small size, with localized thickening of the gallbladder wall, cystic degeneration due to tumor epithelial cells secreting mucin [22] or necrosis, or the presence of multiple gallstones. Five cases from the literature [8, 15, 25, 36, 52], which had either negative primary gallbladder tumorous lesions or benign imaging findings, included three cases with 1-cm sized neoplasms only and two with localized thickening of the gallbladder wall including one case with both wall thickening and small cystic degeneration. Under such circumstances, PET-CT in one patient with thickened walls showed a heterogeneous enhanced mass with FDG accumulation (FDG-avid) in the gallbladder fossa, suggestive of gallbladder cancer [36].

The use of radionuclide 18FDG to diagnose MiNENs of the gallbladder has high sensitivity and specificity, but 18FDG-PET/CT can also lead to false-negative results for well-differentiated NENs [50]. 18FDG-PET/CT may highlight the accumulation of 18FDG in the mass for the gallbladder NEC with the effective clinical diagnosis, and therefore, has been useful for identifying the origin of lymph node metastases [54]. Case two in our case report showed intense FDG uptake in the gallbladder mass. Additionally, functional radiographical imaging such as somatostatin receptor (SSR) imaging with PET can be used to diagnose and differentiate NETs from gallbladder cancer. Since most NETs hold the characteristics of overexpression of SSR on the cell surfaces, radionuclide-labeled somatostatin analogs can tightly bind to SSR for receptor-dependent metabolic changes detected by PET-CT. For example, somatostatin receptor scintigraphy (SRS) uses 111In-octreotide for staging and diagnosing gallbladder NECs. PET-CT with 68Ga-DOTA-NOC as an alternative to SRS can show a hypermetabolic mass [46]. Also, this has the advantages of spatial resolution and better sensitivity and is a faster procedure.

For more obscure imaging findings, biopsy with histological examination should be the last resort. Fine needle aspiration (FNA) biopsy is usually performed under the guidance of either percutaneous ultrasound or CT [8, 14]. In addition, EUS-guided transmucosal FNA is another option [36], which can significantly improve the diagnostic sensitivity to 90% from 74% for EUS alone [35]. It should be emphasized that biopsy is only applied to confirm the diagnosis and not for early diagnosis.

The standard management of early-stage MiNENs of the gallbladder is the same as that for gallbladder cancer, involving radical cholecystectomy, that is, cholecystectomy with en bloc resection of the liver parenchyma surrounding the gallbladder bed and hepatoduodenal ligament lymphadenectomy, and liver segmentectomy is recommended for patients with locally advanced disease [50]. Additionally, simple cholecystectomy is also recommended for early-stage gallbladder NETs such as the T1N0 stage [35]. Pathological stage pT2 and localized liver invasion pT3 gallbladder cancers are suitable for extended radical cholecystectomy [55]. In the present review, among 58 cases of MiNENs of the gallbladder treated by surgical intervention, except for 14 cases of simple cholecystectomy and 4 cases of palliative surgery, the remaining 40 patients in the case reports all underwent radical resection with different ranges of resection according to the degree of tumor progression. Radical cholecystectomy including hepatic segmentectomy seems to improve the 5-year overall survival rate [35]. All six cases of gallbladder MiNENs in Table 2 that had a wide range of local infiltration were treated by extended radical cholecystectomy. Either hepatopancreaticoduodenectomy or pancreaticoduodenectomy was performed in four cases with hepatopancreatic metastasis and did improve the prognosis of these four patients. Although the number of cases was small and the approach could not be statistically compared with other methods, these cases showed that complete resection of the tumor tended to prolong survival [14, 25, 28, 32, 42], and for gallbladder NECs, patients with unresectable masses have a poor prognosis even when treated with chemotherapy and radiation therapy [44].
Neoadjuvant and adjuvant chemotherapies have been proposed as the initial management choice even for surgically resectable cases. Considerable evidence supports the effectiveness of platinum-based drug regimens in the treatment of SCNEC, and this treatment also may be suitable for LCNEC. However, no randomized clinical trials are showing superior efficacy compared with the alternative strategies used for non-neuroendocrine cancers [53], and solid clinical evidence remains lacking for the long-term survival benefit of the regimens. Despite all of this, surgical treatment with adjuvant chemotherapy has been advocated as the putative paradigm for NECs, and postoperative chemotherapy is recommended for advanced stages. However, because MiNEN is rarely sporadic in clinical practice, no general agreement has been reached regarding whether patients with MiNEN should receive chemotherapy, due to the fact that a poor overall response rate has been observed with drugs such as doxorubicin, 5-fluorouracil, cisplatin, and streptozocin alone or in combination [20]. Even though some studies claim that adjuvant chemotherapy may potentially improve the survival of NEC patients [35], a minority only received postoperative chemotherapy or multimodal therapy (21%) for gallbladder NETs, and 70% of patients with gallbladder NETs did not receive any additional therapy after surgery [4]. In the present review, only 15 of 58 patients (25.9%) with gallbladder MiNENs who received postoperative chemotherapy did not show any prolongation in their survival time.

Somatostatin analogs, as a new anti-NEN modality that possesses the effects of anti-tumor proliferation, inhibition of tumor angiogenesis, and promotion of tumor apoptosis, have been used to treat patients with confirmed somatostatin receptor expression on the surface of tumor cells through inhibition of the secretion of a variety of hormones by binding to the somatostatin receptors [35, 50]. Biologic therapies such as long-acting octreotide or lanreotide are able to prolong the overall survival of patients with metastatic mid-gut NEN and ameliorate their symptoms [35, 47]. Neoadjuvant chemotherapy combined with somatostatin successfully converted unresectable MiNEN cases to ones that could be treated by radical resection [35], indicating chemotherapy combined with somatostatin analogs might exert a therapeutic benefit for the long-term prognosis of MiNEN patients.

About one-third of patients had elevated CEA, while more than half of patients were found to have elevated CA19–9. In addition, AFP-producing gallbladder cancer is very rare. The pathological cause of elevated AFP in the gallbladder with MiNEN remains unclear; however, AFP-producing gallbladder cancer is prone to hepatic metastasis and has a poor prognosis [37].

This literature review carried a significant limitation. Since the cases from the literature were not consecutive, and the data extracted from the cases were heterogeneous, it could be impossible to conduct important studies like the prognosis study for long-term assessment of the patients with the disease. In general, such studies presumably require a stringent follow-up by our groups by sending out a questionnaire to each of the patients from the case reports, which could not be done in the reality.

In conclusion, about one-half of patients with MiNENs of the gallbladder, as an extremely rare disease with female predominance, mainly presented with the symptoms of cholelithiasis in the early stage. Preoperatively, the patients might be found to have lymph node metastasis and liver invasion, thus, contrast-enhanced CT, MRI, and 18FDG or 68Ga-DOTA-NOC PET-CT possessed superior value for establishing the diagnosis and planning the treatment choices for NENs. Besides these, either percutaneous or EUS-guided biopsy might also be an effective diagnostic alternative. Essentially, characteristic microscopic cell morphology-findings, Syn and/or CgA expression detected by immunohistochemical staining, NSGs observed by electron microscopy, and NEC and adenocarcinoma components each constituting ≥30% of a neoplasm provided evidence for the patho-histological diagnosis of gallbladder MiNEN. Therapeutically, extended radical cholecystectomy and either adjuvant or neoadjuvant chemotherapy combined with somatostatin analog treatment could be used to treat patients with advanced disease, however, a detailed prognosis analysis should be conducted before claiming the treatments could be beneficial for MiNENs.

Abbreviations
MiNEN: Mixed neuroendocrine–non-neuroendocrine neoplasms; NEC: Neuroendocrine carcinoma; NSE: Neuron-specific enolase; CEA: Carcinoembryonic antigen; AFP: Alpha-fetoprotein; CT: Computed tomography; MRI: Magnetic resonance imaging; ERCP: Endoscopic retrograde cholangiopancreatography; LCNEC: large cell neuroendocrine carcinoma; MRCP: Magnetic resonance cholangiopancreatography; 18FDG-PET: 18F-fluorodeoxyglucose-18F-fluorodeoxyglucose positron emission tomography; Syn: Synaptophysin; CgA: Chromogranin A; TP53: Tumor protein 53; EUS: Endoscopic ultrasonography; ERC: Radical cholecystectomy; SCNEC: small cell neuroendocrine carcinoma; MCN: Multilocular cystic tumor; CI: Confidence interval; PAC: Postoperative adjuvant chemotherapy; ICNP: Intrahepatic cholangiocarcinoma; NEN: Neuroendocrine neoplasm; PET: Positron emission tomography; WHO: World Health Organization; NSG: Neurosecretory granules; SSR: Somatostatin receptor; SRS: Somatostatin receptor scintigraphy; FNA: Fine-needle aspiration.
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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**
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**Consent for publication**
Written informed consent was obtained from the patient’s next of kin for publication of this report and any accompanying images.

**Competing interests**
The authors declare that they have no competing interests.

**Author details**
1. Digestive Hospital of Heilongjiang Provincial Hospital Affiliated to Harbin University of Technology, No. 405, Guoqeli Street, Harbin 150001, Heilongjiang, China. 2. Department of Pathology, Heilongjiang Provincial Hospital Affiliated to Harbin University of Technology, Harbin 150001, Heilongjiang, China. 3. Department of General Surgery, Heilongjiang Provincial Hospital Affiliated to Harbin University of Technology, Harbin 150001, Heilongjiang, China. 4. Department of Gastroenterology, Heilongjiang Provincial Hospital Affiliated to Harbin University of Technology, Harbin 150001, Heilongjiang, China. 5. Digestive Endoscopy Center, Heilongjiang Provincial Hospital, Harbin, China. 6. Department of Pathology, Heilongjiang Provincial Hospital Affiliated to Harbin University of Technology, No. 405, Guoqeli Street, Harbin 150001, Heilongjiang, China. 7. Department of Pathology, Heilongjiang Provincial Hospital, Harbin, China. 8. Department of Gastroenterology, Heilongjiang Provincial Hospital, Harbin, China. 9. Hospital Information Center of Heilongjiang Province Affiliated to Harbin Institute of Technology, Harbin 150001, Heilongjiang Province, China.

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