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Pathological diagnosis, differential diagnosis and origin investigation of easily misdiagnosed adult gastric duplication cysts

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Summary. Objectives. To study diagnosis, differential diagnosis, and origin of easily misdiagnosed adult gastric duplication cysts.

Methods. Six cases with GDCs were studied using immunohistochemical methods to research the expression of tumor markers.

Results. Most GDCs were located in the abdominal cavity outside the digestive organs. The cyst wall tissues included a repetition of the full-thickness structure of the stomach wall that was lined by a variety of different epithelia. The expression of CK7 was positive in all epithelia (6/6), while CK7 expression was positive in cardia glands in 5/5 and positive in fundus glands in 1/5. CK 20 was 100% (4/4) positively expressed in the SCE, 100% (3/3) negative in the CCE, and in the SE. It was also expressed positively 100% (5/5) in fundus glands and 80% (4/5) in cardia glands. Both CK7 and CK20 were negatively expressed in the pyloric glands in case 3. The expression of MUC1 was positive in all epithelia and glands, whereas MUC2 expression was negative. MUC5AC was expressed differently in epithelia and glands.

Conclusions. GDCs can originate from the antrum or the fundus of the stomach. Tumor markers can help diagnosis and differential diagnosis. This study may help to improve clinical precision treatment.

Key words: Gastric duplication cysts, Pathology, Origin, Immunohistochemistry, Differential diagnosis, Precision treatment

Introduction

Gastric Duplication Cysts (GDCs) are a rare type of congenital disease characterized by deformed duplication of the gastrointestinal tract. GDCs occur primarily in newborns and in childhood, usually before the age of 12. The patients typically have symptoms of abdominal pain, vomiting, or weight loss, and a few present with obstruction and perforation of the digestive tract (Kim et al., 2000; Kinugasa et al., 2020; Deesomsak et al., 2013; Namdaroglu et al., 2017). Adults are rarely diagnosed with GDCs, even though there are a few reports. The lack of defining clinical symptoms and the absence of specific imaging and ultrasound results can easily result in misdiagnosis. This will lead to overly extensive surgery and severely affect patients' life quality due to overtreatment. Here, we study the clinicopathological characteristics and expression characteristics of tumor markers in six cases of GDCs. Our results provide guidance for improved diagnosis and differential diagnosis and referential experiences for precise treatment.

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Materials and methods

Patient information

Six cases of GDCs in this study were collected from March 2011 to May 2021 in the Department of Pathology in Peking University People's Hospital. The patients included five female and one male. The ages ranged from 22 years old to 67 years old. The size of the surgical specimens ranged from 1.2 cm to 13.6 cm. All the above information is complete and the data is effective to do the research. This study was approved by the hospital Ethics Review Committee.

Methods

The approach of immunohistochemistry was adopted to conduct this study. After collection, the specimens were sectioned for H&E staining using routine pathological methods. Immunohistochemistry was performed using EnVision, a two-step method. The antibody to CK7 was purchased from Beijing Jinqiao Yatu Biotechnology Co., Ltd., China; while CK20, MUC1, MUC2, MUC5AC, AAT, GATA-3, and Alpha-

Antitrypsin (AAT) were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., China. CK7, CK20, MUC1, MUC2, MUC5AC, AAT were expressed in the cytoplasm, whereas GATA3 was expressed in the nucleus. The positive reaction presented as brown-yellow color.

A summed score (AxB) was calculated based on the proportion (A) and intensity (B) of positively stained tumor cells. For A, 1 point was assigned if <25% of tumor cells were positively stained, 2 points if 25–50%, and 3 points if >50%. Zero (0) point was assigned if there were no positively stained tumor cells. For B, 1 point was assigned for light yellow staining intensity (weak), 2 points for brownish-yellow intensity (intermediate), and 3 points for tan coloration (strong). If the sum of AxB was <4, the staining result was considered negative, whereas a sum of ≥4 was considered positive.

Results

Clinical characteristics

In six cases of GDCs, the male to female ratio was 1:5, the median age at presentation was 41 years (range from 22 to 67 years old). All cases were divided into 10-year-old intervals. Three cases (50%) were between 20-29 years old, while from the ranges of 40-49, 50-59, and 60-69 years old, there was one case respectively. By comparison, there was no case in the range of 30-39 years old, which suggests that the onset of adult GDCs is concentrated more on the range of 20-29 years of age. All cases were accidentally found in the abdominal cavity during physical examinations. Two cases (cases 1 and 4) occurred in the stomach wall; case 2 in the retroperitoneum above the left kidney; case 5 in the posterior peritoneum of the pancreatic body and tail; case 3 in the hepatogastric space on the lesser curvature of the stomach; and case 6 in the abdominal cavity of the lower-left liver. The clinical symptoms were non-specific, with occasional abdominal pain, bloating, or upper abdominal discomfort. There was no vomiting, diarrhea, fever, jaundice, or weight change. All cases of imaging (Fig. 1) CT showed a cystic mass or low-density shadows. There was no obvious enhancement of the cyst wall on enhanced CT. Case 5 was further enhanced by MRI. Further MRI enhancement in case 5 showed the cystic lesion with enhanced surrounding tissues,which was considered benign. Two cases of EUS with laparoscopic partial gastrectomy indicated a round mass on the stomach wall, with uneven internal echo, partially hypoechoic, without flow blood, considered as GIST. Laparoscopic partial gastrectomy was performed on two cases of GDCs located in the stomach and one case in the hepatogastric space on the lesser curvature of the stomach. Two cases (cases 2 and 6) underwent simple cystectomy, and pancreatic body tail excision and splenectomy were performed in case 5. There was no recurrence after regular follow-up.

Pathological features

Mass examination

Case 1. A 55-year-old female was suspected to have a GIST based on CT and EUS examination. The abdominal cavity was explored during the operation, and an exogenous mass was seen near the cardia on the side of the lesser curvature of the stomach fundus. The size was 3.2×2.4 cm, with clear borders and an intact

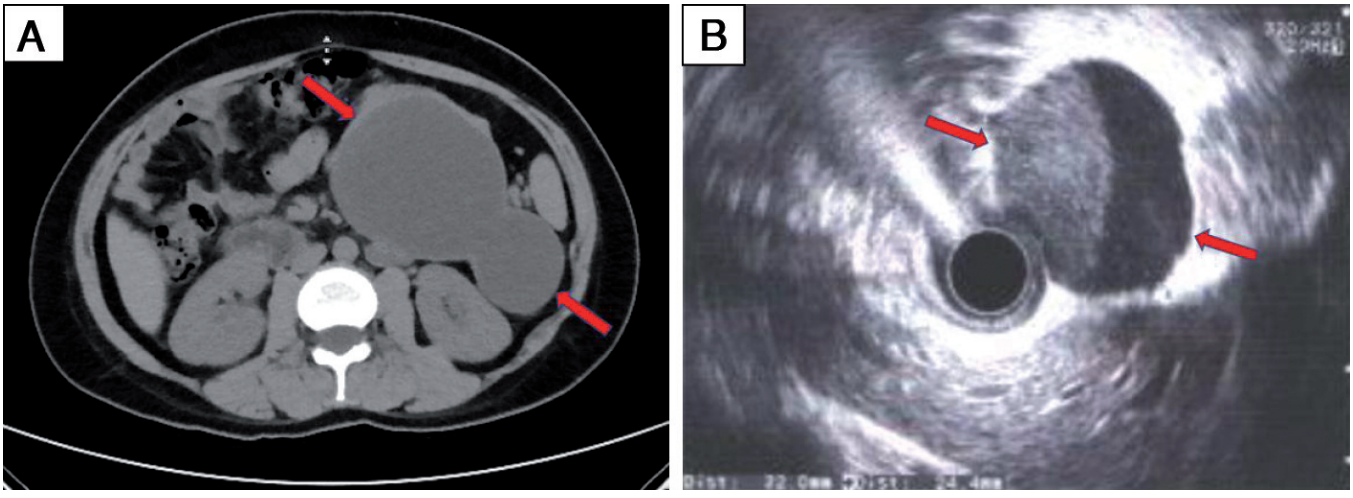

Fig. 1. Imaging characteristics of GDCs. **A.** Computer tomography imaging of the abdomen and pelvis from case 5. A huge cystic mass in the back of the pancreas body and the spleen and kidney space (red arrowheads). **B.** EUS of case 4 showed a round-like mass on the stomach wall with uneven internal echo (red arrowheads).

capsule. The cut surface of the tumor was cystic, with a tough texture, containing a transparent clear liquid. The capsule wall had a uniform texture and the surface was smooth.

Case 2. A 26-year-old woman had a retroperitoneal cyst above the left kidney, 9.0×5.2 cm in size. The cyst was closely related to the lesser curvature of the stomach and contained dark red liquid. There were folds on the inner wall, which was smooth and 0.3-0.5 cm thick, without obvious protrusions or nipples.

Case 3. A 22-year-old female had a hepatogastric space-occupying mass on the lesser curvature of the stomach. CT and MRI examinations revealed a cyst, the diameter of which was 4 cm, which contained a chocolate jelly-like substance. The wall thickness was 0.1-0.2 cm, with smooth inner and outer walls and the inner wall had no papillary protrusions or thickened areas.

Case 4. A 46-year-old female had a gastric fundus tumor. There were gray nodules on the stomach wall, 2.0×1.2 cm in size, with clear boundaries. The tumor was located in the mucosal layer of the stomach wall. The cut surface was cystic and solid, and the texture of solid areas were soft.

Case 5. A 28-year-old female had a retroperitoneal cyst behind the body and tail of the pancreas (Fig. 2). With a clear boundary, the tumor had a uniform wall thickness of 0.2-0.3 cm. It grew in a dumbbell-shaped swelling shape with a size of 13.2×8.1 cm. The tumor contained soy milk-like liquid and was accompanied by a double spleen. Case 6. A 67-year-old male had a raised inner wall of the common bile duct. A free cyst was seen below the left liver. The size was 8×3 cm, containing white mucus. The cyst wall had no inner wall nodules or nipple protrusions and was smooth.

Microscopic examination

Most cyst walls of the GDCs were composed of the mucosal layer of the stomach (partially visible gastric glands), muscularis mucosa, submucosa, muscular layer, and subserosa. Case 1. The full-thickness structure of the stomach wall was observed on the cyst wall. The surface was lined by ciliated columnar epithelium (CCE), the simple columnar epithelium (SCE), and a small amount of squamous epithelium (SE). Cardia glands (CGs) and fundus glands (FGs) were visible.

Case 2. The full-thickness structure of the stomach wall was seen on the cyst wall, lined by CCE and SCE, which was not intraepithelial neoplasia. CGs and FGs were seen under the epithelium, accompanied by inflammatory cell infiltration, peripheral blood vessel dilation, and congestion.

Case 3. The full-thickness structure of the stomach was seen on the cyst wall. The surface was lined by CCE and a small amount of SE. No intraepithelial neoplasia was seen, and PGs were seen under the epithelium.

Case 4. The cystic wall was lined by SCE, CGs and FGs were seen under the epithelium. Ulcer formation and epithelial loss were noted in some areas.

Case 5. The full-thickness structure of the stomach wall was observed on the cyst wall, lined by SCE and a

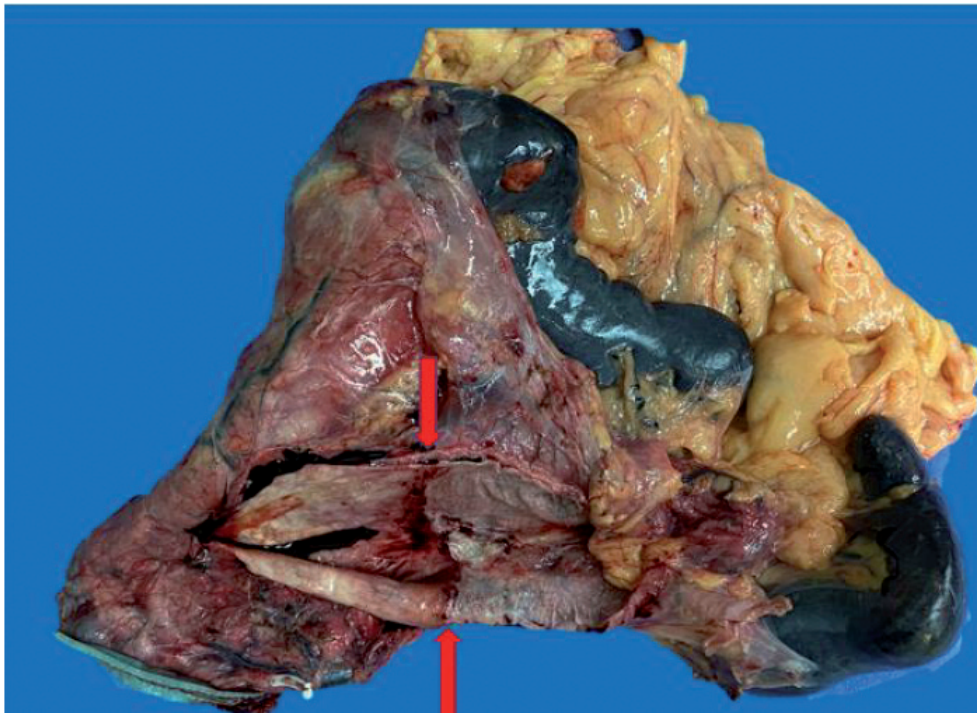

Fig. 2. Cut section of the resected specimen of a GDC. A retroperitoneal cyst was behind the body and tail of the pancreas. The inner and outer walls were smooth without papillary protrusions and thickened areas (red arrowheads, case 5).

small amount of SE. No intraepithelial neoplasia was seen. CGs and FGs were visible, accompanied by inflammatory cell infiltration.

Case 6. The cyst wall was full-thickness tissue of the stomach wall, lined by SCE. CGs and FGs were seen under the epithelium, and there was no evidence of cancer tissue invasion. In addition, there was a poorly differentiated adenocarcinoma of common bile duct eminence, with tumor thrombus in the vessel, and cancer metastasis in the lymph nodes around the bile duct wall (2/2).

See Fig. 3 and Table 1 for a summary of details.

Immunohistochemistry

Case 1. The surface of the cyst wall was lined by CCE, SCE, and a small amount of SE: CK7 and MUC1 (all epithelia and glands +). MUC2 (all epithelia and glands -). CK20 (CCE and SE-, SCE+; CGs+, FG luminal border +), MUC5AC (all epithelia +, CGs+, FGs-).

Case 2. The cyst wall was lined by CCE and SCE: CK7, MUC1, and MUC5AC (all epithelia and glands +). MUC2 (all epithelia and glands -). CK20 (CCE-, SCE+; CGs-, FG luminal border +). In this case, the tumor was located on the posterior peritoneum above the left kidney. In order to distinguish it from a kidney-derived cyst, the expression of GATA-3 was tested, and the result was negative.

Case 3. The surface of the cyst wall was lined by CCE and a small amount of SE, CK7 (all epithelia+, PGs -), MUC1 (all epithelia and glands +), MUC2 and CK20 (all epithelia and glands -), MUC5AC (all epithelia-, PGs +).

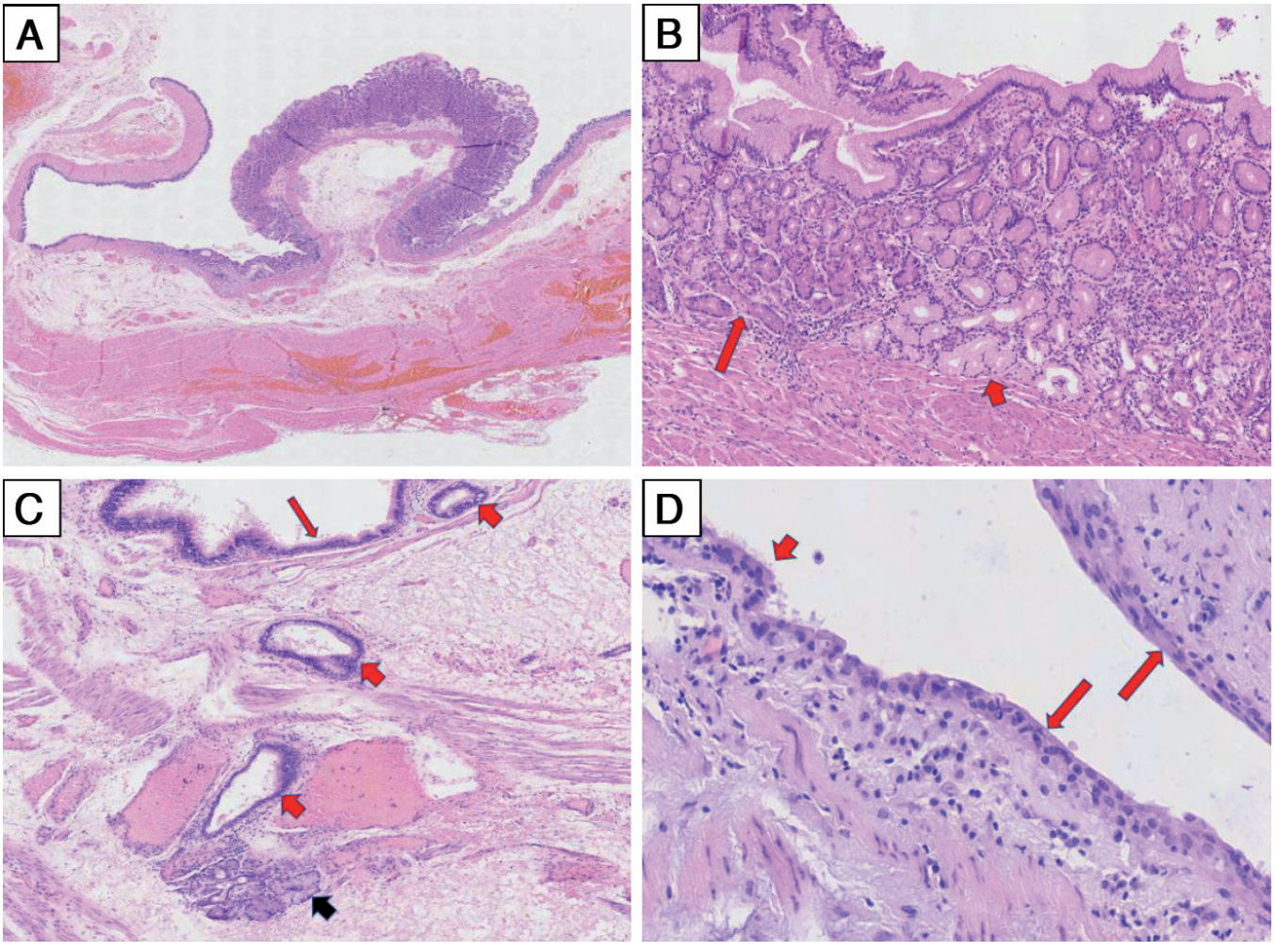

Fig. 3. Microscopic examination of GDCs. **A.** Low power view of GDC from case 2 composed of the mucosal layer of the stomach, muscularis mucosa, submucosa, muscular, and subserosa. HE. **B.** Both CGs (short arrowhead) and FG (long arrowhead) were seen in case 6. HE. **C.** Low power view from case 3 showed the cyst wall was lined by CCE (red arrowhead) and Pyloric glands (black arrowhead). HE. **D.** The higher power view of case 1 showed the cyst wall was lined by PCCE (short arrowhead) and SE (long arrowheads). HE. A, C, x 40; B, x 100; D, x 200.

Case 4. The cyst wall was lined by SCE, CK7 (SCE +, CGs + and FGs -), MUC1, MUC5AC and CK20 (all epithelia and glands +), MUC2 (all epithelia and glands -). Case 5. The cyst wall was lined by SCE and a small amount of SE, CK7 (SCE+, SE+, CGs +, FGs -), CK20 (SCE+, SE-, CGs +, FGs luminal border +), MUC1 (all epithelia and glands +), MUC5AC (SCE+, SE-, CGs +, FGs luminal border +), MUC2 (all epithelia and glands -). In this case, the tumor occurred in the retroperitoneum behind the body and tail of the pancreas. It needed to be differentiated from a primary pancreatic cyst, and AAT immunohistochemical staining was performed. The result was negative.

Case 6. The cyst wall was lined by SCE: CK7 (SCE+, CGs +, FGs -), CK20 and MUC1 (All epithelia and glands +), MUC5AC (SCE+, CGs+, FGs -), MUC2 (all epithelia and glands -).

The immunohistochemical staining results of all cases are shown in Fig. 4 and Table 1.

Discussion

Gastrointestinal duplication cysts include gastric (GDCs) and intestinal duplication cysts, which can occur in any part of the digestive tract from mouth to rectum, but often occur in the ileum. GDCs account for about 2-8% of duplication cysts, arising in the early embryonic stage and typically presenting before 12 years old. Duplication cysts are believed to occur before the epithelial cells of the gastrointestinal tract are fully differentiated, with abnormal recanalization after normal development of the stomach (Chan et al., 2018). McLetchie believed that adhesion between the notochord and embryonic endoderm may not extend as quickly as its surrounding structures, leading to traction diverticula and the formation of repeated cysts (Mcletchie et al., 1954). Other theories include aborted twin embryos, persistent embryonic diverticula, and repetitive deformities caused by hypoxia or traumatic events. None of these claims can prove and explain all gastrointestinal duplication cysts (Napolitano et al., 2013; Chan et al., 2018). According to literature studies, GDCs can be divided into cystic and tubular, depending on their connectivity and proximity to the stomach. About 80% of the gastric duplication was cystic and did not communicate with the gastric cavity. The remaining 20% consisted of tubular GDCs, which were adjacent to the stomach and communicated with the gastric cavity to some extent2. GDCs were mostly located near the greater curvature of the stomach because during development, these duplications were usually distributed on the dorsal side of the original intestine (Napolitano et al., 2013). About 5.5% of cases were located on the lesser curvature of the stomach. The mechanism of their occurrence was not clear.

Adults with GDCs are relatively rare. Women are slightly more susceptible (about twice as much) than men. The prevalence of GDC in men and women in this study was 1:5 and was more common in young women (3/6). GDCs are often found accidentally. A small number of patients had symptoms such as abdominal pain, bloating, vomiting, or upper abdominal discomfort (Aranburu et al., 2015). We speculate that a small number of GDCs without symptoms in neonates and childhood are eventually discovered accidentally in adulthood. Some scholars have proposed that the three diagnostic criteria for gastric duplication cysts are (Mansour et al., 2020): (1) the wall of the cyst is adjacent to the gastric wall; (2) smooth muscle can be seen around the cyst which is connected to the gastric muscle layer; (3) the cyst wall is lined by the gastric or intestinal epithelium. However, through careful

Table 1. Clinicopathological characteristics and the expression of tumor markers of six cases GDCs.

Case	Age	Gender	Location	Imaging	Epithelium and Glands	CK7	CK20	MUC1	MUC2	MUC5AC
1	55	Female	Lesser curvature of the fundus near the cardia	EUS: Round mass	CCE	+	-	+	-	+
					SCE	+	+	+	-	+
					SE	+	-	+	-	+
					CG	+	+	+	-	+
					FG	-	Luminal border+	+	-	-
2	26	Female	Retroperitoneum above left kidney	Enhanced CT: Low density shadow	CCE	+	-	+	-	+
					SCE	+	+	+	-	+
					CG	+	-	+	-	+
					FG	+	Luminal border+	+	-	+
3	22	Female	Hepatogastric space on the side of lesser curvature of stomach	Enhanced MRI: Cystic mass	CCE	+	-	+	-	-
					SE	+	-	+	-	-
					PG	-	-	+	-	+
4	46	Female	Fundus of stomach	EUS: Uneven echo	SCE	+	+	+	-	+
					CG	+	+	+	-	+
					FG	-	Luminal border+	+	-	+
5	28	Female	Pancreatic body and tail behind retroperitoneum	CT: Cystic mass MRI: Cystic enhancing mass	SCE	+	+	+	-	+
					SE	+	-	+	-	-
					CG	+	+	+	-	+
					FG	-	Luminal border+	+	-	+
6	67	Male	The abdominal cavity of the lower left liver	Unknown	SCE	+	+	+	-	+
					CG	+	+	+	-	+
					FG	-	Luminal border+	+	-	-

US, endoscopic ultrasound; CT, computer tomography; MRI, magnetic resonance imaging.

observation, all cases in this study showed that the entire stomach wall tissue was duplicated (Fig. 3A), including the mucosal layer, muscularis mucosa, submucosa, muscular layer, and the serous layer of the stomach. Gensler et al proposed that respiratory epithelium may appear in gastric duplication cysts, suggesting that foregut undifferentiated epithelium may transit to differentiated specialized epithelium during the embryonic period (Gensler et al., 1966). GDCs are thought to be the foregut duplications (FDCs) of the stomach when pseudostratified ciliated columnar epithelium reveals foregut duplication of the stomach. Most FDCs are located in the upper part of the stomach, near the gastroesophageal junction at the level of the cardia, or the anterior or posterior wall of the stomach fundus (Napolitano et al., 2013). In our study, case1, case 2 and case 3 showed the structure of pseudostratified ciliated columnar epithelium, so they can be called FDC. However, only the GDC of case 1 was located near the cardia on the side of the lesser curvature of the stomach fundus, Case 2, and case 3 occurred outside the digestive organs. In this study, 4/6 cases of GDCs were located in the abdominal cavity outside the digestive organs, to which great importance should be attached for all surgeons and imaging physicians.

Generally, glands in different parts of the stomach have certain differences in the expression of tumor markers: most of the gastric antrum is pyloric glands, and some glands are lined by cilia. The expression characteristics of pyloric glands are all CK7, CK20, and MUC2 negative, but both MUC1 and MUC5AC positive. Most of the body and the fundus of the stomach are fundus glands, and a small number of the cardia glands are in the fundus of the stomach. The expression characteristics of cardia glands are all CK7, CK20,

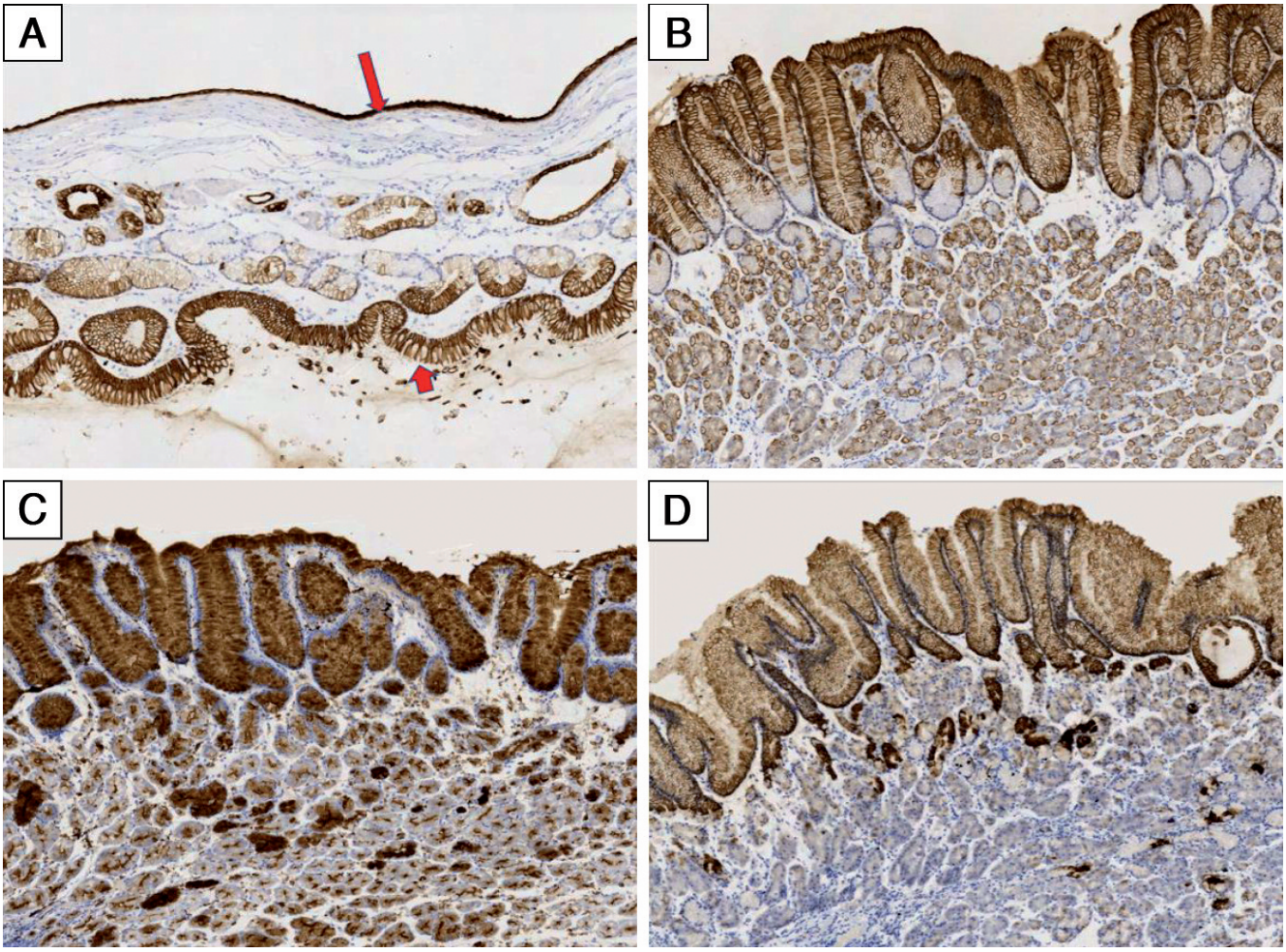

Fig. 4. Immunohistochemistry of GDCs in case 5. **A.** CK7 was positive in SCE (short arrowhead), SE (red arrowhead), and MGs, EnVision. **B.** CK20 was positive in SCE, CGs, and FG (luminal border), EnVision. **C.** MUC1 was positive in SCE (diffuse cytoplasmic), CGs, and FG, EnVision. **D.** MUC5AC was positive in SCE, CGs, and FG (weak), EnVision. x 100.

MUC1, and MUC5AC positive, MUC2 negative and the expression characteristics of gastric fundus glands are CK7-, MUC2 and MUC5AC all negative, CK20 and MUC1 positive. Case 3 contained the pyloric glands, and the expression characteristics of the markers were consistent with the gastric antrum. Therefore, we consider that the GDC of case 3 is a repetitive cyst originating from the gastric antrum. The remaining 5 cases contained fundus glands and cardia glands. The expression characteristics of the markers were basically similar to those of the stomach fundus. We speculated that they were derived from repeated cysts in the fundus of the stomach. There is no GDC that simply contains fundus glands. Therefore, there is no evidence that GDCs originate from the gastric corpus. No similar reports have been made in the world before.

Imaging has limited significance in the diagnosis of this disease, and may even lead to misdiagnosis. In this study, there were two cases of gastric duplication cysts that occurred in the non-digestive system that needed to be differentiated from primary cysts in adjacent tissues and organs. For example, case 5 was misdiagnosed as pancreatic mucinous cystadenoma by imaging examination. AAT is a marker specific to the pancreas. In this case, the staining result was negative, which ruled out the diagnosis of pancreatic mucinous cystadenoma and other pancreatic primary cystic tumors. However, according to the imaging report and considering that the cyst was larger, the Whipple operation in this case seriously affected the patient's quality of life. Interestingly, this case was also accompanied by polysplenia deformity. Previously, it has been reported in the literature that GDCs can coexist with multiple spleens. This congenital malformation may be due to the same embryonic origin of the two organs from the dorsal mesangium of the stomach. The most typical concurrent deformity was polysplenia with short pancreas (Passos et al., 2017; Hu and Gui, 2019). In our case, no congenital developmental abnormalities, such as short pancreas, were found. Attention should be paid to the coexistence of gastrointestinal duplication malformations and other congenital malformations. If congenital abnormalities such as polysplenia or short pancreas are found, further examination should be made for other malformations. The remaining five cases did not exhibit congenital abnormalities of the spleen. The pathogenesis of GDCs still needs more exploration and research. Another case reported here occurred in the posterior peritoneum above the left kidney. GATA-3 is specifically expressed in tumors derived from the kidney and breast. The expression was negative in this case, which ruled out the diagnosis of a renal primary cyst. Imaging is often confused with the diagnosis of gastrointestinal stromal tumors (GIST). GISTs can occur in any part of the gastrointestinal tract. About 54% of GISTs occur in the stomach. Most GISTs grow in a nodular shape. Hemorrhagic necrosis and cystic degeneration can be seen on the cut surface. These tumor nodules are mainly composed of spindle cells under the microscope (Nagtegaal et al., 2020). It was reported in the literature that GDCs can be accompanied by GISTs (Lee et al., 2020), with single or multiple small nodules in the cyst wall. In this study, two cases of GDCs (cases 1 and 4) occurred in the stomach with maximum diameters of 2cm and 3.2cm, respectively. However, GISTs were suspected by imaging, and EUS, gastroscopy, and partial gastrectomy were performed. The expansion of the scope of surgery may affect the patient's long-term quality of life. In recent years, some scholars recommended EUS-guided fine needle aspiration biopsy before surgery. EUS can show the hyperechoic mucosa and hypoechoic smooth muscle layer inside the cyst (Abdulla et al., 2017). If ciliated columnar epithelial structures are observed under the microscope, an experienced pathologist may consider whether there was abnormal development of the gastrointestinal tract. However, some scholars have argued that regardless of whether there were GDCs in the EUS-FNA, surgical removal of the lesion should be considered firstly. We had two cases of GDCs located in the stomach wall that underwent EUS for GIST. After complete resection, the pathological final diagnosis of GDCs was completely different from the diagnosis of preoperative EUS.

Complications of GDCs include obstruction, bleeding, repeated episodes of pancreatitis, and malignant transformation. Malignant transformation of GDCs is extremely rare. The most common histological type is adenocarcinoma7. Individual cases have noted malignant transformation to squamous cell carcinoma, neuroendocrine tumors, and GIST (Barussaud et al., 2008). Once the malignant transformation of GDCs occurs, the prognosis is poor, and it can relapse and metastasize. In this study, SE was found in the lining epithelium in two cases, but no malignant manifestations were seen. So far, there are no cases of GDCs occurring with cholangiocarcinoma in the literature. One of our cases was GDC with cholangiocarcinoma. Are these two types of lesions single, or are adenocarcinomas developed from GDCs? In the previous literature on malignant transformation of GDCs, atypical glands infiltrated and grew in the cyst walls, and invaded the smooth muscle layer (Kang et al., 2014; Yamasaki et al., 2016). In our study, common bile duct adenocarcinoma and GDCs occurred in different locations. There was no correlation Between GDCs and bile duct adenocarcinoma. Some scholars proposed that intraoperative frozen pathological diagnosis can provide histological diagnosis and intraoperative surgical guidance for the malignant transformation of GDCs (Geng et al., 2015).

GDCs in newborns and children often manifest as respiratory and gastrointestinal symptoms. The diagnosis depends on auxiliary examinations such as imaging and ultrasound followed by surgical treatment, and the postoperative pathological diagnosis is confirmed (Carachi and Azmy, 2002). However, adult GDCs are usually clinically asymptomatic. According to the latest literature (Kim et al., 2021), ESD was an alternative

treatment for GDCs and was suitable for small lesions in the submucosa of the gastric wall. However, many surgeons, imaging doctors, and pathologists have not understood this disease yet. It is very difficult to make a correct diagnosis by imaging, and misdiagnosis easily leads to excessive surgery. This study found that the cyst wall of GDCs under the microscope was composed of the entire structure of the stomach wall. It was the first time that we pointed out the pathological morphology of the subserosa membrane of GDCs.We propose that the combined application of immunohistochemistry can help distinguish GDCs from different primary organs. With the application of EUS-FNA preoperative diagnosis or intraoperative frozen diagnosis can assist in exclusion of malignant transformation, avoiding misdiagnosis, over-treatment, and thereby improve quality of life.

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Competing interests. The authors declare that there are no conflicts of interest.

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