

## CASE REPORT

## Gallbladder carcinoma – a rare cause of pyloric-duodenal stenosis

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### Abstract

Pyloric duodenal stenosis is usually caused by pyloric, juxtapyloric or duodenal ulcer, or by postbulbar ulcer. Gallbladder cancer (GBC), duodenal diverticula, annular pancreas and superior mesenteric artery syndrome (Wilkie's syndrome) are rare causes of pyloric duodenal stenosis. The case of a 66-year-old female patient is presented. The patient was admitted to hospital presenting anorexia, repeated alimentary vomiting, epigastric pain, and weight loss. Objective clinical examination upon admission: clapotage à jeun is present, triggered by tapping the epigastric region. Laboratory tests reveal moderate anemia, hypokalemic alkalosis, increased levels of cholestatic enzymes and of tumor markers. Gastroendoscopy: Stomach presenting stasis fluid in large quantity. Deformed antropyloric region caused by extrinsic compression. Abdominal native magnetic resonance imaging (MRI) and with contrast medium: cholecyst lumen entirely obstructed with calculi; thickened wall, with heterogeneous gadolinophilia; gadolinophilic mass erasing the bordering limit in relation to the cholecyst wall and the colon hepatic angle, and leaving a print on the pyloric region. During surgery, upon opening the peritoneal cavity, a tumoral pericholecystic block was observed, including the pyloric-duodenal region and the transverse mesocolon. Histopathology tests of tissue samples showed adipose conjunctive tissue with invasive adenocarcinoma. Immunohistochemical tests [cytokeratin (CK) 7, CK17, CK19, CK20, CDX2, mucin (MUC) 1, MUC2, MUC5AC, MUC6, epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA)] were consistent with infiltrating neoplastic carcinoma, originating in the gallbladder epithelium. Gastrointestinal obstruction cases caused by gallbladder carcinoma are rare. The pyloric-duodenal region is more frequently affected, as compared to the small intestine or the colon.

**Keywords:** gallbladder carcinoma, pyloric duodenal stenosis, adenocarcinoma, immunohistochemistry.

### Introduction

Pyloric-duodenal stenosis is usually caused by pyloric, juxtapyloric, duodenal, or postbulbar ulcer. Duodenal diverticula, annular pancreas, superior mesenteric artery syndrome (Wilkie's syndrome) or gallbladder cancer are uncommon causes. Friedman, Mehler and Ginzburg described the first case of pyloric-duodenal obstruction caused by gallbladder carcinoma, published in the *American Journal of Gastroenterology* (1969).

Gallbladder cancer, though generally considered rare, is the most common malignancy of the biliary tract, accounting for 80–95% of biliary tract cancers [1]. The majority of cases are diagnosed in the advanced stages, leading to extremely poor prognosis. The prognosis is mainly dependent on histological subtype, grade, and stage of the tumor at the time of presentation. The overall mean survival rate for patients with gallbladder cancer (GBC) is six months, with a five-year survival rate of 5%.

In most instances, gallbladder cancer develops over 5 to 15 years, when metaplasia progresses to dysplasia, carcinoma *in situ*, and then invasive cancer [2]. Gender differences demonstrate a marked predominance of women over men worldwide. Women are affected two to six times more often than men. The importance of female sex hormones is increased because gallbladder cancer is associated with a higher rate and greater number of pregnancies [3, 4].

Maximum incidence occurs over the age of 50 [2]. Incidence rates are high in Latin America and Asia, relatively high in some countries in Eastern and Central Europe (Hungary, Germany, Poland, and Romania), yet low in the United States and most Western and Mediterranean European countries (UK, France, and Norway) [3]. Increased stone size augments the risk of gallbladder cancer; stones >3 cm carry a 10-fold increased risk when compared with smaller stones. The basis for this relationship likely resides in gallstones creating local mucosal irritation and chronic inflammation, perhaps aided by the local production of carcinogens, such as secondary bile acids [5–8].

Adenocarcinoma is the most frequent histological type, accounting for 98% of all gallbladder tumors; two-thirds of these are moderately or poorly differentiated. The other histopathological variants include the papillary, mucinous, squamous, and adenosquamous subtypes [6, 9, 10].

Since gastrointestinal obstruction caused by gallbladder cancer has been rarely described, the current presentation is considered pertinent.

### Case presentation

A 66-year-old patient was admitted (2013) in the Medical Clinic II, "St. Apostle Andrew" Emergency County Hospital, Constanta, Romania, presenting anorexia, repeated alimentary vomiting, epigastric pain, and weight loss.

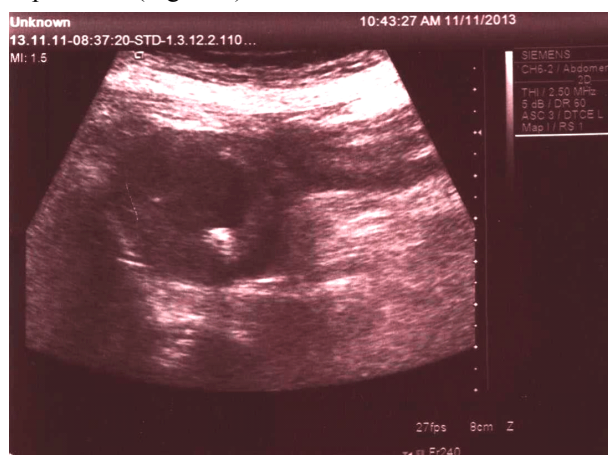
Patient history recorded insidious onset symptoms, consisting of initially moderate epigastric pain irradiating in the right hypochondrium and alimentary vomiting; at first, vomiting was followed by pain relief and ameliorated general state. Abdominal pain gradually becomes colicative and vomiting is recurrent, abundant, and foul smelling; vomiting is not followed by improved pain symptoms and it rapidly triggers denutrition and dehydration.

Absence of digestive pathology history in this patient is to be noted, as well as the fact that the patient denies alcohol consumption and smoking.

Objective clinical examination upon admission: normally colored, dry, and inelastic teguments and mucosa. Clapotage à jeun is present, triggered by tapping the epigastric region. Epigastric and periumbilical region is bulging, with intermittent tonic contractions projected at the abdominal wall level. After nasogastric aspiration, all these signs disappear. Muddy biliary fluids are present on gastric probe. Diuresis 1000 mL/24 h, blood pressure (BP) 90/60 mmHg, atrio-ventricular (AV) rhythm 100 beats/min.

The paraclinical tests revealed: hemoglobin (Hb) 10 g/dL, leukocytes (L)  $10\,500/\text{mm}^3$ , hypokalemic alkalosis [ $\text{K}^+$  2.8 mmol/L, respiratory alkalosis (RA) 37 mEq/ $\text{CO}_2$ ],  $\text{Na}^+$  130 mmol/L, glycemia 128 mg/dL, serum creatinine 1.31 mg/dL, urea 41 mg/dL, creatinine clearance 50 mL/min, total bilirubin (TB) 0.64 mg/dL, alkaline phosphatase (ALP) 251 U/L,  $\gamma$ -glutamyltransferase (GGT) 349 U/L, cancer antigen (CA) 19-9 2645 U/mL, carcinoembryonic antigen (CEA) 2.2 ng/mL.

Abdominal ultrasound: gastric antrum with thickened wall, mainly in anterior position, and presence of stasis fluid. D2 cross section reveals uneven wall thickening and minimum remaining lumen. Cholecyst is entirely occupied by calculi, with thickened parcellar wall and hypoechogenic irregular cauliflower-like mass infiltrating the wall. There is weak demarcation of surrounding tissue, especially the hepatic one (Figure 1).



**Figure 1** – Abdominal ultrasound (cholecyst entirely occupied by calculi, with thickened parcellar wall and hypoechogenic irregular cauliflower-like mass infiltrating the wall, gastric antrum with thickened wall, mainly in anterior position, and presence of stasis fluid).

Gastroendoscopy: Stomach containing large quantity of stasis fluid. Deformed antropyloric region caused by extrinsic compression. Pyloric visualization and passage to duodenum are not possible.

Abdominal native computed tomography (CT) and with contrast medium: thick and irregular cholecyst lumen wall presenting a pseudo-nodular pattern in certain places (maximum 41/32 mm axial diameters on ventral versant of corpus–fundus region); tumor-like CT aspect with predominantly low-uptake heterogeneous iodophilia. Structural changes of gallbladder fundus region do not present fatty cleavage plane as to visceral side of hepatic segments IV and V, as to duodenal bulb and pylorus (with compressive effect at this level, with gastric dilation upstream); it involves colon hepatic flexure joining at this level. Missing parcellar principal biliary duct (PBD) visualization with dilation upstream of intrahepatic biliary ducts is to be noted – observation: PBD tumor invasion. Small oval-shaped 5 mm hyper-density at gallbladder level, attached to corporal region ventral wall – calculus (Figure 2).

Abdominal native magnetic resonance imaging (MRI) and with contrast medium: cholecyst lumen entirely obstructed with 10–35 mm diameter calculi; thickened wall of  $\approx 15$  mm in circumference, presenting heterogeneous gadolinophilia. Gadolinophilic mass of  $\approx 48/36$  mm closely placed at the bordering limit in relation to the cholecyst wall and the colon hepatic angle, leaving an extrinsic print on the pyloric region. PBD  $\approx 7$  mm, imprinted extrinsically. No imaging suggestive of adenopathy (Figures 3 and 4).

Anamnesis corroborated with paraclinical data suggests diagnosis of gallbladder neoplasm with loco-regional invasion – pyloric-duodenal stenosis and colon hepatic flexure invasion.

Surgery: upon surgical opening of peritoneal cavity, a pericholecystic tumor mass was observed which incorporated the pyloric-duodenal area and transverse mesocolon. Hepatic pedicle was invaded by tumoral adenopathy. Small-size hepatic metastases, disseminated on both hepatic lobes, were observed. Post-stenotic dilated stomach; adenopathy was present at lesser curvature and mesenteric levels. Pericholecystic hepatic tumor extension was present.

Under the circumstances, biopsy was decided and practiced on two hepatic nodes, from the lesser stomach curvature and hepatic pedicle; since local conditions excluded retrogastric transmesocolic anastomosis, gastro-jejunal anastomosis was performed.

Our patient developed respiratory failure following surgery, was intubated and placed on ventilation support. After several days on mechanical ventilation, she was taken off ventilation at her family's request and died.

Histopathological examination was performed in the Department of Anatomopathology, “St. Apostle Andrew” Emergency County Hospital, Constanța. The slides were examined with Leica DM750 microscope and image captured with Leica ICC50 HD, at high resolution.

Conventional histopathological method was used on microscopic samples made of 5- $\mu\text{m}$  formalin-fixed paraffin-embedded tissue sections that were routinely deparaffinized in toluene and rehydrated in ethanol-graded baths, and then stained with Merck's Hematoxylin–Eosin (HE) and Periodic Acid–Schiff (PAS).

Histopathological examination of sample tissue fragments (from small curvature ganglia, tumoral extension, and hepatic pedicle ganglia) revealed conjunctive and adipose tissue presenting carcinoma-type tumor prolifera-



ration, disposed in cell groups, isolated cells, and poor delineated tubes with eosinophilic cytoplasm, and pleomorphic nuclei, with tachychromasia and inconspicuous nucleoli; some clear/foamy intracytoplasmic mucin-like

vacuoles were noticed; tumor architecture was solid/pseudoglandular, and with tumor presence in lymphovascular spaces (Figures 5 and 6). PAS staining revealed fine PAS-positive intracytoplasmic drops.



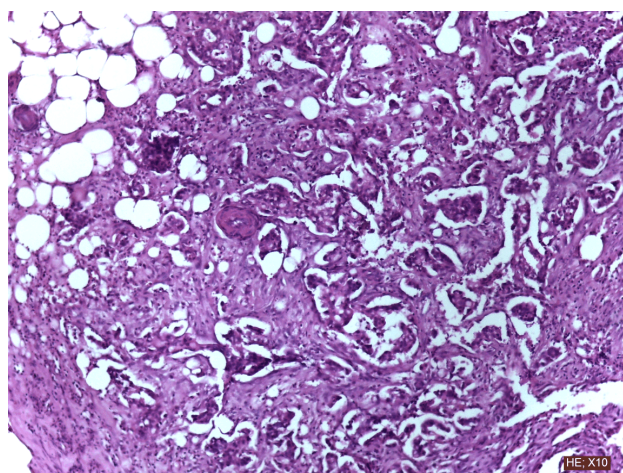
**Figure 2 – CT with contrast medium (thick and irregular cholecyst lumen wall presenting a pseudo-nodular pattern in certain places with low-uptake heterogeneous iodophilia; gastric wall with thickened wall and presence of stasis fluid).**



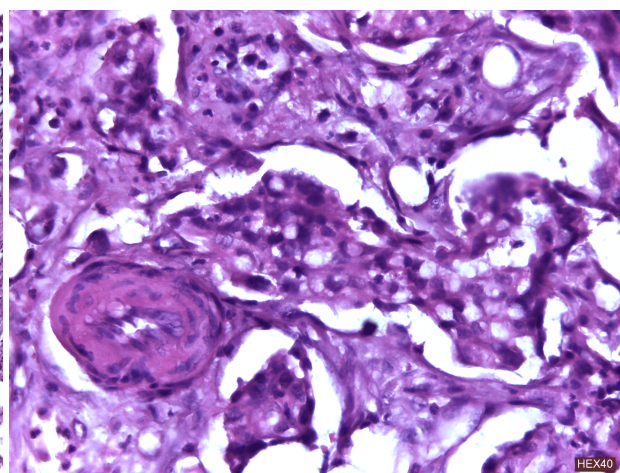
**Figure 3 – Abdominal MRI native: coronal T2 sequence (thickened cholecyst wall).**



**Figure 4 – Abdominal MRI with contrast medium: axial T1 fat sat sequence (cholecyst thickened wall presenting heterogeneous gadolinophilia; gadolinophilic mass placed at the bordering limit in relation to the cholecyst wall and the colon hepatic angle).**



**Figure 5 – Islands and poorly designed glandular tubes of tumor cells, with eosinophilic cytoplasm, a lot of them in lymphovascular spaces, with obvious nearby location to an arteriole. Some intracytoplasmic clear/foamy vacuoles are noticed. HE staining, ×100.**



**Figure 6 – High-power view to see lymphovascular spaces with tumoral emboli and periarteriole invasion. Clear/foamy mucin-like cytoplasmic vacuoles are seen. Nuclei are pleomorphic, with variable chromasia and inconspicuous nucleoli. HE staining, ×400.**

Immunoperoxidase studies were performed on 4-μm formalin-fixed paraffin-embedded tissue sections that were routinely deparaffinized in toluene and rehydrated in ethanol-graded baths. All sections were pretreated with Dako Retrieval Solution, code 2369/citrate buffer (pH 6) and water bath immersion at 99°C (20 minutes in; 20 minutes out). Primary antibody source and dilutions were as follows: cytokeratin (CK) 7 (DAKO; clone OV-TL 12/30; code IR619, ready-to-use); CK20 (DAKO; clone Ks20.8; code IR777, ready-to-use); CK17 (DAKO; clone E3, code IR620, ready-to-use); CK19 (DAKO; clone RCK108, code IR615), CDX2 (DAKO; clone DAK-CDX2, code IR080, ready-to-use), CEA (DAKO, polyclonal, code IR526, ready-to-use), epithelial membrane antigen (EMA) (DAKO, clone E29, code IR629, ready-to-use), mucin (MUC) 2 (DAKO, clone CCP58, ready-

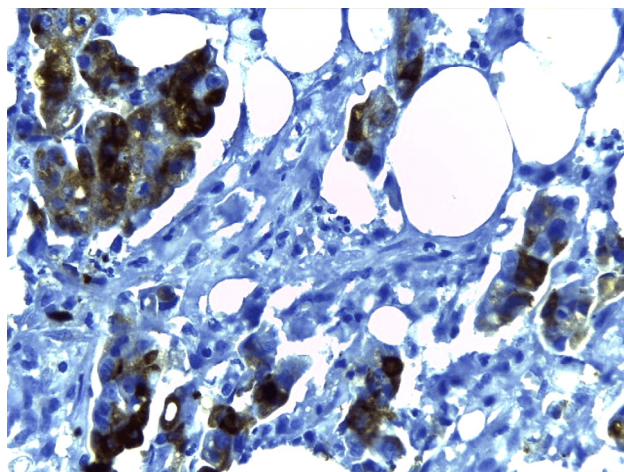
to-use), MUC5AC (DAKO, clone CLH2, code IR661, ready-to-use), MUC6 (clone CLH5, Novocastra), p53 (DAKO, clone DO-7, code IR616, ready-to-use), alpha-methylacyl-CoA racemase (AMACR) (DAKO, clone 13H14, code IR060) and hepatocyte (DAKO, clone OCH1E5, code IR6240). Visualization was performed with DakoEnVision™+/HRP (Horseradish peroxidase) kit, code 4004. Appropriate external control was used. Nuclear staining was assessed for p53, CDX2, membranous staining for EMA, cytoplasmic and membranous staining for CEA, whereas cytoplasmic staining was evaluated for CK7, CK17, CK19 and CK20, MUC1, MUC2, MUC6, MUC5AC, AMACR and hepatocyte. Immunoreactivity was evaluated according to the intensity of tumor cell staining (0–3+), as well as according to the percentage of tumor cells that were stained (minimum 10% for



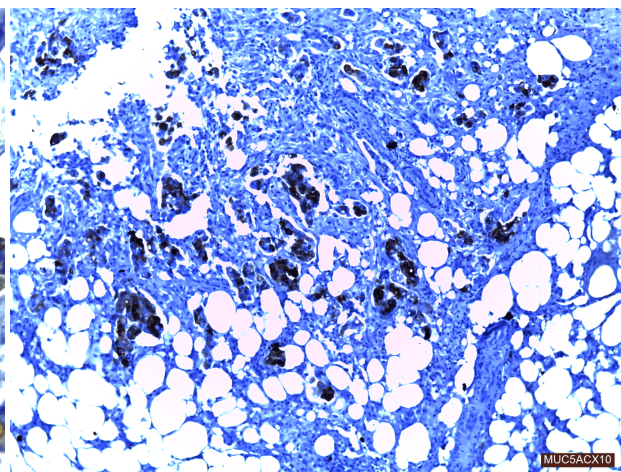
positive reaction, less than 50% from all tumor for focal reaction, more than 50% for diffuse reaction).

The immunohistochemical (IHC) tests presented: CK7 – positive (+) cytoplasmic, focally in tumor cells; CK20 – negative in tumor cells (no cell reactivity); CK19 – negative in tumor cells (no cell reactivity); CK17 – positive (++) cytoplasmic in tumor cells (Figure 7); EMA – positive (+++) membranar, intensely diffuse in all tumor cells; CEA – positive (+++) cytoplasmic and membranar,

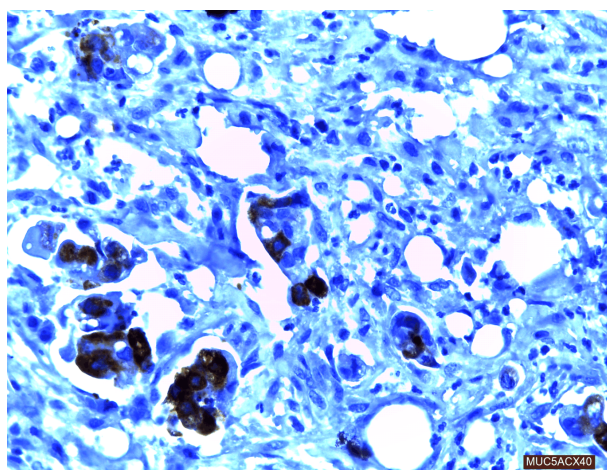
intensely diffuse in all tumor cells; MUC1 – positive (+++) cytoplasmic, intensely diffuse in 80% of tumor cells; MUC2 – negative (no cell reactivity); MUC5AC – positive (+++) cytoplasmic, intensely diffuse in 60% tumor cells (Figures 8 and 9); MUC6 – negative in tumor cells; CDX2 – negative in tumor cells; P53 – positive (+) in <10% of tumor nuclei; AMACR – moderate/intense positive (+++/+++) reaction, cytoplasmic, focal; hepatocyte – negative reaction.



**Figure 7 – Intense cytoplasmic positive reaction for CK17. IHC staining for CK17, ×400.**



**Figure 8 – Diffuse positive reaction for MUC5AC, over 50% of the tumor. IHC staining for MUC5AC, ×100.**



**Figure 9 – High-power view of the previous image. IHC staining for MUC5AC, ×400.**

## Discussion

Gallbladder cancer is the most frequent biliary malignant tumor, presenting two major associations: biliary calculi and vesicle adenoma. It has been reported that a large stone size of more than 3 cm, a family history of GBC, and prolonged cholelithiasis are potential risk factors for GBC [11–13]. These factors could be used in decision making when performing a cholecystectomy for asymptomatic gallstones. However, no definite evidence of direct causal relationship between gallstones and gallbladder cancer has been presented and biases of other risk factors remain unsolved problems [14].

Gallbladder adenomyomatosis has not been considered to have malignant potential; however, several reports have suggested that gallbladder cancer may originate from

adenomyomatosis [15]. Recently, a study showed that gross features of adenomyomatosis were found in approximately a quarter of gallbladders resected under the diagnosis of GBC [16].

Other risk factors recently receiving attention include bacterial infections. Although supporting evidence for an association is weak, *Salmonella* [17, 18] and *Helicobacter* species [19] would be prime candidates for a bacterial predisposition to GBC.

No specific clinical elements are associated to early-stage cancer. Dyspeptic symptoms or colic caused by biliary lithiasis, acute cholecystitis signs, or icterus may occur. Icterus presence indicates unresectable stage; however, icterus may also be caused by calculi migration and carcinoma may be resectable.

Gallbladder carcinomas (GBCs) frequently show vascular invasion and metastasis when carcinoma cells invade the perimuscular connective tissue (pT2 according to TNM classification) through the muscular layer. Two intramural invasion patterns were defined as infiltrative growth (IG) type, infiltrative growth in the muscle layer without destruction, and destructive growth (DG) type, massive growth with destruction of the muscle layer [20].

The DG type was significantly associated with poor differentiation, aggressive infiltration, and decreased post-operative survival in terms of its histological differentiation, lymphatic invasion, venous invasion, lymph node status, neural invasion, and mode of subserosal infiltration. Radical resection of advanced GBC is sometimes difficult because of frequent lymph node metastasis.

Presence of abdominal tumor, ascites, or duodenal obstruction suggests unresectability [21].

Gastrointestinal obstruction cases triggered by the gallbladder carcinoma are uncommon. Cases of small intestine or colon obstruction have been described in

medical literature [16]; however, the pyloric-duodenal area is more commonly affected, although the incidence in most series is low [3, 16].

Paraclinical explorations are non-specific. In advanced stages, these tests actually reflect biliary tract obstruction: serum cholestasis and bilirubin levels are raised. High ALP and GGT serum levels are possible, even in non-icterus patients – as in our patient's case; however, this increase does not indicate non-resectable stage, but merely vesicular-bed invasion, cholangitis, or hepatic-duct unilateral obstruction.

No specific tumor-marker has been identified for biliary-duct carcinoma. However, certain markers are more frequently associated with this type of carcinoma. Assay of CA242, CA15-3, CA19-9 and CA125 are fairly good markers for discriminating between patients with gallbladder carcinoma and patients with cholelithiasis [22].

CA19-9 is a sensitive marker, although moderately high levels are also found in benign biliary complaints. Very high levels ( $>1000$  U/mL), as in the case of our patient (2645 U/mL), are registered in malignant disease.

CEA is also raised in patients with gallbladder carcinoma [12]. In our case, however, CEA level was normal.

CA242 is a promising tumor marker for GBC and performs better than CEA and CA19-9. CA242 and CA125, when used together, achieved best sensitivity and specificity. Serum markers seem to be less sensitive when used individually in carcinoma of the gallbladder but may prove useful in combination.

Most gallbladder carcinomas (60–90%) develop in the presence of calculi. The presence of large gallstone is one of the major risk factors. A stone size of more than 3 cm, a family history of GBC, and the duration of cholelithiasis are potential risk factors for developing GBC [23].

Chronic gallbladder trauma and inflammation caused by calculi, which leads to dysplasia, is probably the link between biliary lithiasis and gallbladder cancer [12, 24].

IHC tests were consistent with neoplastic infiltration of poorly differentiated adenocarcinoma not otherwise specified (NOS), originating in pancreaticobiliary tract or superior digestive tract (owing to MUC1+/CK17+ reaction) [25].

Pancreatic origin was then excluded, owing to CK19 negativity and clinic/imagistic. Gastrointestinal origin was excluded too, on account of CDX2/CK19/CK20/MUC2 negativity and MUC1+/CK17+ reaction [25]. Hepatic carcinoma was excluded because of CK17 positivity [26].

Gallbladder origin was eventually established owing to AMACR positivity [27].

The MUC5AC positive reaction was explained by the presence of mucin in the cytoplasm of tumor cells, as expressed also by the PAS+ reaction.

The MUC6 loss of expression may be attributed to tumor cell invasivity [28].

The wild type P53 reaction, in our case, was not surprising; it was noticed in other research studies of biliary tract cancers and is not a discriminatory factor [29–31].

## Conclusions

The patient admitted in our hospital presented a clinical status suggestive of pyloric stenosis and the gastroendo-

scopy highlighted a stomach filled with stasis fluid in large quantities and the antropyloric area deformed due to extrinsic compression. Further investigations (abdominal ultrasound, CT, MRI) have revealed a gallbladder cancer with locoregional invasion and pyloroduodenal stenosis, a rare complication described in the literature. Pyloric-duodenal area is more frequently affected, as compared to small intestine or colon. Presence of duodenal obstruction, abdominal tumor or ascites, suggests unresectability. Paraclinical explorations were non-specific. High ALP and GGT serum levels are possible, even in non-icterus patients – as in our patient's case; however, this increase does not indicate non-resectable stage, but merely vesicular-bed invasion, cholangitis, or hepatic-duct unilateral obstruction. Tumor markers have an increasing significance in the diagnosis and evaluation of GBC. Assays of CA242, CA15-3, CA19-9, and CA125 are fairly good markers for discriminating between patients with carcinoma of the gallbladder and patients with cholelithiasis. As concerns histopathology and immunohistochemistry, this case was a poorly differentiated adenocarcinoma, originating in the gallbladder, excluding hepatic, pancreatic and gastrointestinal mucosa origin. The pancreaticobiliary origin was readily clear, but the gallbladder origin was eventually established owing to AMACR tumor positive reaction. Neither histological grading nor T factor correlated with mucin or p53 expression, respectively. The MUC6 loss of expression may be attributed to invasivity of tumor cells.

## Consent

Written informed consent was obtained from the patient's next of kin for publication of this case report and any accompanying images.

## Conflict of interests

The authors declare that they have no conflict of interests.

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