Tumor of the Gallbladder in a dog

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Signalement:

An 11 year-old neutered female Boston Terrier dog.

Clinical History:

The dog was presented for an acute episode of vomiting and hematemesis, few hours after ingestion of bones. Polydipsia and polyphagia had been also observed by the owners for several months.

Clinical findings:

The dog was alert and presented a moderate abdominal enlargement associated with a potbellied appearance, a moderate hepatomegaly and a ventral thin and easily wrinkled skin. Hypertension was also noticed (systolic blood pressure: 180 mmHg).

Diagnostic procedures:

Complete blood cell count, biochemical & hemostasis panel and urinalysis were performed. Results are in **Tables 1, 2, 3** and **4**.

The complete blood cell count revealed a slight normocytic normochromic regenerative anemia associated with a moderate thrombocytosis (**Table 1**). Some signs of regeneration were noticed on the blood smear, with a moderate

anisocytosis and polychromasia, a slight metarubricytosis and Howell Jolly bodies. These abnormalities could be most probably consistent with blood loss.

The biochemical panel revealed a mild hyperprotidemia possibly secondary to dehydration or an inflammatory process, a slight hypochloremia consistent with vomiting, and a mild to moderate increase in the liver enzyme activities including ALT, ALP and GGT most probably consistent with cholestasis and/or an induction of these enzymes, and maybe moderate cell damage (**Table 2**).

The urinalysis revealed a low USG and dipstick revealed a marked proteinuria. A bacterial culture was negative, and the urine sediment was unremarkable. A renal proteinuria was suspected, and a "cause and effect" relationship between proteinuria and hypertension was suspected at this stage (**Table 3**).

The hemostasis results were within reference range (**Table 4**).

An abdominal ultrasonography was not suggestive of a digestive foreign body, and revealed a large intraluminal mass within the gallbladder associated with a diffuse and moderate hepatomegaly and a bilateral hypertrophy of adrenal glands (**Figure 1).**

The gallbladder mass was uppermost investigated because of its potential involvement in the acute clinical signs and a fine needle aspirate was performed (**Figure 2**). The cytological evaluation revealed a moderate cellularity, a hemorrhagic background associated with a blue-gray finely granular substance highly suggestive of bile fluid and numerous sometimes giant naked nuclei. The few intact cells were dispersed or clustered in unorganized clumps, and the nuclei were often embedded in a cytoplasmic background. The intact cells were round to polygonal with a medium NCR, and were characterized by a medium to slight basophilic cytoplasm containing some to many fine azurophilic granules, and a round to oval nucleus associated with a fine chromatin with sometimes one nucleolus of variable size (small to large). These cells presented some criteria of malignancy with a marked anisocytosis and anisokaryosis, some binucleations and macronucleolations. These cells had features of a neuroendocrine tumor, and a carcinoid tumor was suspected. A biopsy and immunohistochemistry were recommended to confirm this hypothesis.

A CT-scan was performed to characterize the gallbladder mass for surgery, and for staging. It confirmed that the mass was attached to the gallbladder wall, confirmed the bilateral hypertrophy of adrenal glands, and revealed very small hepatic nodules of undetermined origin. A cholecystectomy was performed, and the multiple hepatic nodules were not macroscopically observed during the laparotomy. The gallbladder mass was infiltrative and exophytic within the

lumen (2 x 3 cm). The histological examination revealed a proliferation of neoplastic cells which formed some small aggregates or nests, sometimes oriented and separated by a fine fibrovascular stroma forming a pseudo-lobular pattern. The neoplastic cells were oval to polygonal with a hyperchromatic round to oval nucleus associated with a small to large nucleolus, and a moderate abundant eosinophilic cytoplasm sometimes granular or vacuolated. These cells revealed some cytological criteria of malignancy (anisokaryosis, anisocytosis, binucleation…) associated with an infiltrative tendency and a medium mitotic index. These features were consistent with a neuroendocrine tumor of the gallbladder such as a well-differentiated neuroendocrine carcinoma (*i.e* a carcinoid tumor). An immunohistochemistry pattern confirmed this hypothesis, including a negative staining of a combination of Cytokeratins (Cytokeratin 5, 6, 8, 17 and 19) and a strongly positive staining of Chromogranin A and Neuron Specific Enolase (NSE).

Treatment and follow-up:

At the follow-up, an ACTH stimulation test was performed to explore a highly suspected hyperadrenocorticism. The exaggerated response to ACTH confirmed a Cushing's syndrome (**Table 2**). A pituitary-dependent hyperadrenocortism (PDH) was suspected as primary hypothesis because of the bilateral and symmetric hypertrophy of the adrenal gland noticed on abdominal ultrasonography and CT scan, but an ectopic ACTH secretion due to the carcinoid tumor could not be completely ruled out. Even if the carcinoid tumor was removed, very few remaining secreting cells in the gallbladder or elsewhere (e.g: in the liver in case of an extension of the tumor) could be sufficient to induce a hyperadrenocorticism.

The hyperadrenocorticism was treated with trilostane, and regular ACTH stimulation tests were performed to adjust the dose. The hypertension was treated with amlodipine. During the follow-up, a progressive decrease in liver enzymes activity was noticed, a proteinuria remained (urine protein: creatinine ratio: between 5 and 7), and a potential metastatic extension to the liver was suspected on abdominal ultrasound examination. A liver fine needle aspirate could not confirm this suspicion and was suggestive of a glucocorticoid hepatopathy, but an adjuvant treatment based on toceranib phosphate and carboplatin was applied because metastases of the primary tumor could not be ruled out. Unfortunately, the dog developed an acute renal failure and a severe gastrointestinal disease during hospitalisation, and the owners decided to euthanize.

Necropsy and histological findings:

At the necropsy, the main gross findings were those of a uremic syndrome (presence of sub-pleural mineralizations and a diffuse congestion and edema of the pulmonary parenchyma) associated with a chronic glomerulopathy and signs of hemorrhagic colitis. Histopathological examination revealed no metastatic extension of the carcinoid. A severe renal amyloidosis, a light lobular hyperplasia of hepatic parenchyma and a cortical hyperplasia of adrenal glands were also noticed. The pituitary gland was autolyzed and no histological lesion could be identified.

Discussion

Neuroendocrine tumors are rare in dogs and cats. They arise from disseminated neuroendocrine cells which produce peptides and bioactive amines in different organs. The most common sites are the lung and the gastrointestinal tract, but other uncommon sites have been reported in dogs, such as the gallbladder, liver, bile duct, skin, nasal cavity and naso-pharynx. Few cases of gallbladder carcinoid have been yet reported [1-4].

The clinical signs and clinical pathologic findings of gallbladder carcinoid are unspecific. The clinical signs include in particular hematemesis and vomiting like in our case, but also lethargy, fever, abdominal pain, icterus, diarrhoea and melena. Upper gastro-intestinal blood loss in case of gallbladder carcinoid could be due to blood loss from the neoplasm [4], and bleeding associated with hemobilia and hemocholecyst have been reported in three previous cases [1,4]. However, a differential diagnosis of upper gastrointestinal ulcers should be considered since bleeding due to ulceration induced by an eventual secretion of gastrin by the neoplastic cells (*i.e* Zollinger-Ellison syndrome) have been described in people and suspected in a cat with a hepatic carcinoid [5]. In our case, the primary hematemesis was suspected to be associated with blood loss from the neoplasm because it had also been induced after the fine needle aspiration, and no evidence of healing gastrointestinal ulcers was noticed at the necropsy. The clinical pathologic findings of gallbladder carcinoid can commonly include increase of various liver enzymes activities such as ALT, AST, ALP, and GGT as in our case, but they can also be the result of Cushing's syndrome (cf. glucocorticoid induction and steroid hepatopathy) [1,4]. However, the observed increase in GGT especially when compared with the magnitude of the increase in ALP could raise a suspicion of biliary tract disease in our case. Anemia, leukocytosis and

increase of bilirubin were also previously reported for gallbladder carcinoid tumor [1, 4]. The persistent proteinuria was initially suspected to be related to the Cushing's syndrome and hypertension in our case, but could also be due to the renal amyloidosis discovered at the necropsy.

The definitive diagnosis of neuroendocrine tumor is based on histologic features associated with immunohistochemical stainings. A relevant panel including Chromogranin A, synaptophysin and NSE is recommended to diagnose hepatic carcinoid [6]. In few cases of reported gallbladder carcinoid, immunohistochemical investigations mainly used a common negative immunoreactivity for cytokeratin, and a positive Chromogranin A [1-4], NSE [3-4], protein gene product 9.5 [1] and synaptophysin [4] immunoreactivity. The Grimelius reaction detecting argyrophilia and the transmission electron microscopy were infrequently used to confirm the neuroendocrine origin of the tumor [2].

Biologic behavior of neuroendocrine tumor is difficult to predict on histological features, since anaplasia features and mitotic index are not reliable to determine the malignancy grade. The evidence of tumor invasion into adjacent tissue could reflect criteria of malignancy. So, it is recommended to consider these tumors as potentially malignant. Hepatic carcinoid has been described as an aggressive tumor associated with a common metastatic potential to the peritoneum and draining lymph nodes [7, 8], however it is suggested that in case of gallbladder carcinoid without evidence of intraperitoneal or distant metastasis, the prognosis is considered as good [4] after a surgical treatment. In our case, the use of an adjuvant treatment based on toceranib phosphate and carboplatin was decided because of the suspicion of a possible metastatic extension of the tumor to the liver. However, no evidence of metastasis has been observed at the necropsy and histological examination.

Neuroendocrine cells may produce various hormones (ACTH, secretin, gastrin, serotonin…), and paraneoplastic syndromes have been described in human medicine due to the release of some biologically active peptides by the neoplastic cells. As an example, the ectopic secretion of ACTH by a neuroendocrine tumor is well recognized in human medicine and especially in case of carcinoid tumors. An ectopic secretion of ACTH due to a hepatic carcinoid and metastasized neuroendocrine tissue in pancreatic, lymph node and liver has been suspected in two dogs [9, 10]. Hypokaliema was a main biochemical finding in these two cases, which is not a main feature of hyperadrenocorticism in dogs. However, hypokalemia is mainly present in case of hyperadrenocortiscism in humans due to an ectopic ACTH secretion. In fact, the concentration of cortisol is commonly higher in case of ectopic ACTH secretion, and a cortisol-induced mineralo-corticoid effect on renal collecting tubule cells has been reported. The high level of cortisol

saturates the renal tubular protective enzyme (11β-hydroxysteroid dehydrogenase) which inactivates cortisol to cortisone, and therefore allows cortisol binding to aldosterone receptors, thus promoting renal potassium excretion [9]. In our case, a hyperadrenocorticism was still suspected after the surgical excision of the gallbladder carcinoid, and was confirmed some days later. The persistence of the hypercortisolism after the cholecystectomy associated with no evidence of metastatic expansion, the progressive improvement of the cortisol concentration and liver enzymes activities with the medical treatment of the Cushing's syndrome, and the absence of hypokalemia make the possibility of an ectopic ACTH secretion unlikely. However this possibility cannot be completely ruled out, since Pituitary-dependent hyperadrenocorticism (PDH) and ectopic ACTH secretion remain very difficult to distinguish. Even if no metastatic expansion of the tumor could be found at necropsy, a very small subpopulation of remaining neoplastic cells may have secreted ACTH. An immunohistochemical staining for ACTH has not been performed. In case of a positive reactivity it could confirm an ectopic ACTH secretion, but a negative result could not exclude it because a secretion of ACTH by the neoplastic cells cannot always be highlighted by immunohistochemistry (peptide hormones could be immediately or episodically excreted, or presence of too few secreting neoplastic cells) [10]. Other tests could be used to try to discriminate PDH and ectopic ACTH secretion: CRH administration which usually induces an increase of plasma ACTH and cortisol concentrations in people and dogs with Cushing's syndrome, rarely induce an increase of plasma ACTH concentration in case of ectopic ACTH secretion in people, and the plasma concentration of ACTH seems to be higher and not suppressible with high dose of dexamethasone in case of ectopic syndrome [10, 11]. These tests could not be performed in our case. Nevertheless, the co-existence of a gallbladder carcinoid and a PDH is more likely in this case.

Conclusion:

This case shows an unusual case of gallbladder tumor, a carcinoid tumor diagnosed by cytology and confirmed by histology and immunohistochemistry. The clinical presentation was characterized by chronic clinical signs consistent with a Cushing's syndrome and acute clinical signs which could be secondary to the neoplastic invasion of the gallbladder. In this case, the differential diagnosis of the Cushing's syndrome should include an ectopic ACTH secretion by the gallbladder carcinoid tumor.

Table 1: Hematology results obtained with the Procyte® (Idexx)

Analytes	Observed value	Reference Interval
HGB (g/dL)	10.9	12.0-18.0
RBC (.10^{12}/L)	4.65	5.50-8.50
HCT (L/L)	0.38	0.37-0.55
MCV (fL)	70.5	60.0-77.0
MCH (pg)	23.4	18.5-30.0
MCHC (g/dL)	33.2	30.0-37.5
PLT-I (.10^{9}/L)	788	175-500
WBC (.10^{9}/L)	12.8	5.5-16.9
Neutrophils (.10^{9}/L)	10.3 (80.7%)	2.0-12.0
Lymphocytes (.10^{9}/L)	1.3 (9.8%)	0.5-4.9
Monocytes (.10^{9}/L)	1.1 (8.8%)	0.3-2.0
Eosinophils (.10^{9}/L)	0.1 (0.5%)	0.1-1.5
Reticulocytes (.10^{9}/L)	139	< 110
Reticulocytes (%)	3.7	-

Table 2: Biochemistry results obtained with the Vitros 350® (Orthoclinical) and *Immulite® (Siemens)

Analytes	Observed value	Reference Interval
Total Proteins (g/L)	72	55-66
Albumin (g/L)	28	23-39
Glucose (mmol/L)	8.0	3.7-8.2
Na (mmol/L)	144	138-148
K (mmol/L)	4.0	3.2-5.0
Cl (mmol/L)	108	110-118
Creatinine (µmol/L)	100	44-133
Total Bilirubin (µmol/L)	4.5	1.7-12.0
GGT (U/L)	106	5-25
PAL (U/L)	475	20-155
ALAT (U/L)	383	3-50
*Cortisol T_0 (nmol/L)	58 .5	-
*Cortisol T_{0+1h} (nmol/L) ACTH stimulation test	877	< 500

Table 3: Urinalysis results

Analytes	Data	Reference Interval
Source	Cystocentesis	-
Color	Clear	Clear yellow
USG	1.020	1.015-1.045
pH	6	6-7.5
Sediment	< 5 cells / 40 PF	< 5 cells / 40 PF
Dipstick	Proteinuria +++	-

Table 4: Hemostasis results obtained with the SAC 2000 analyzer (Synbiotics)

Analytes	Data	Reference Interval
PT (s)	10	12-17
aPTT (s)	79	71-102

Figure 1: Ultrasonography of the liver and the gallbladder. Presence of an infiltrative mass in the wall of the gallbladder (within the yellow circle line)

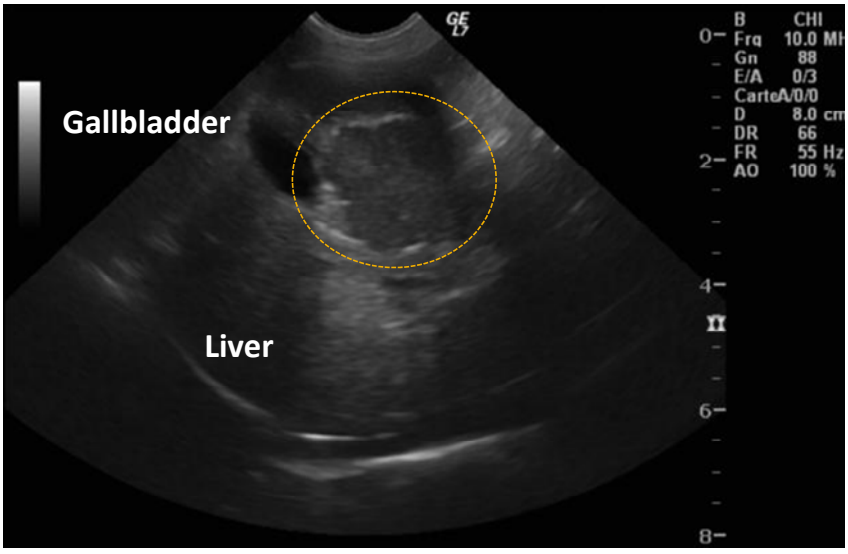


Figure 2: Cytology of the gallbladder's mass (modified May-Grünwald Giemsa staining). **A & B**: Moderate cellularity with a hemorrhagic background associated with a blue-gray finely granular substance highly suggestive of bile fluid, numerous naked nuclei embedded in a cytoplasmic background and few intact round to polygonal cells. **C & D**: Cells with moderate medium to slight basophilic cytoplasm containing fine azurophilic granules, and a round to oval nucleus with a fine chromatin sometimes associated with one or two nucleoli. Presence of some criteria of malignancy: marked anisocytosis and anisokaryosis. These cells have features of a neuroendocrine tumor, and a carcinoid tumor is more likely.

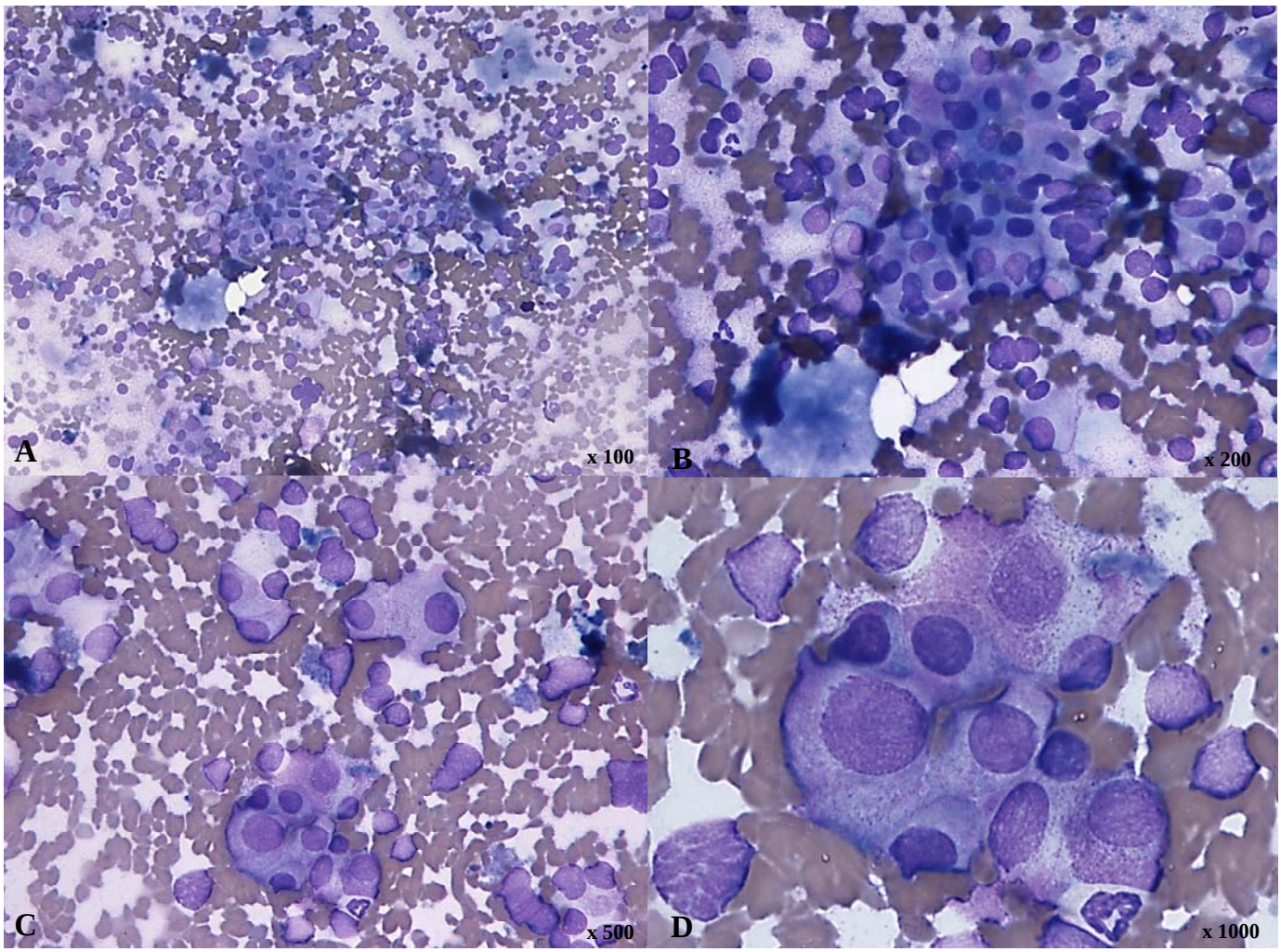


Figure 3: Gross morphology of the gallbladder's mass after surgical excision. Presence of an infiltrative and exophytic mass within the lumen of the gallbladder (2 x 3 cm).

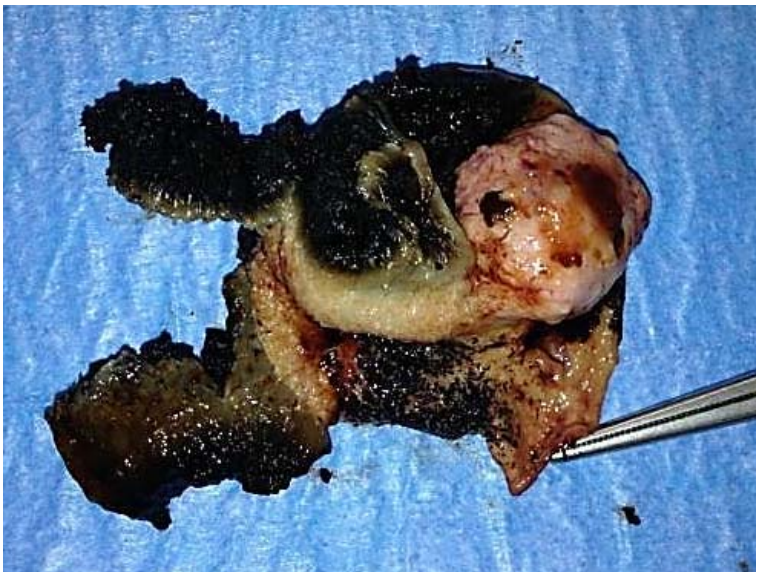


Figure 4: Histology of the gallbladder's mass (HE staining). **A & B**: Proliferation of neoplastic cells which forms some small aggregates or nests, sometimes oriented and separated by a fine fibrovascular stroma forming a pseudo-lobular pattern. **C & D**: The neoplastic cells were uniform, oval to polygonal, with a hyperchromatic round to oval nucleus associated with a small to large nucleolus, and a moderate abundant eosinophilic cytoplasm sometimes granular or vacuolated. These cells revealed some criteria of malignancy: marked anisocytosis and anisokaryosis, some binucleations, and a medium mitotic index. These features were consistent with a neuroendocrine tumor of the gallbladder, and a carcinoid tumor is more likely.

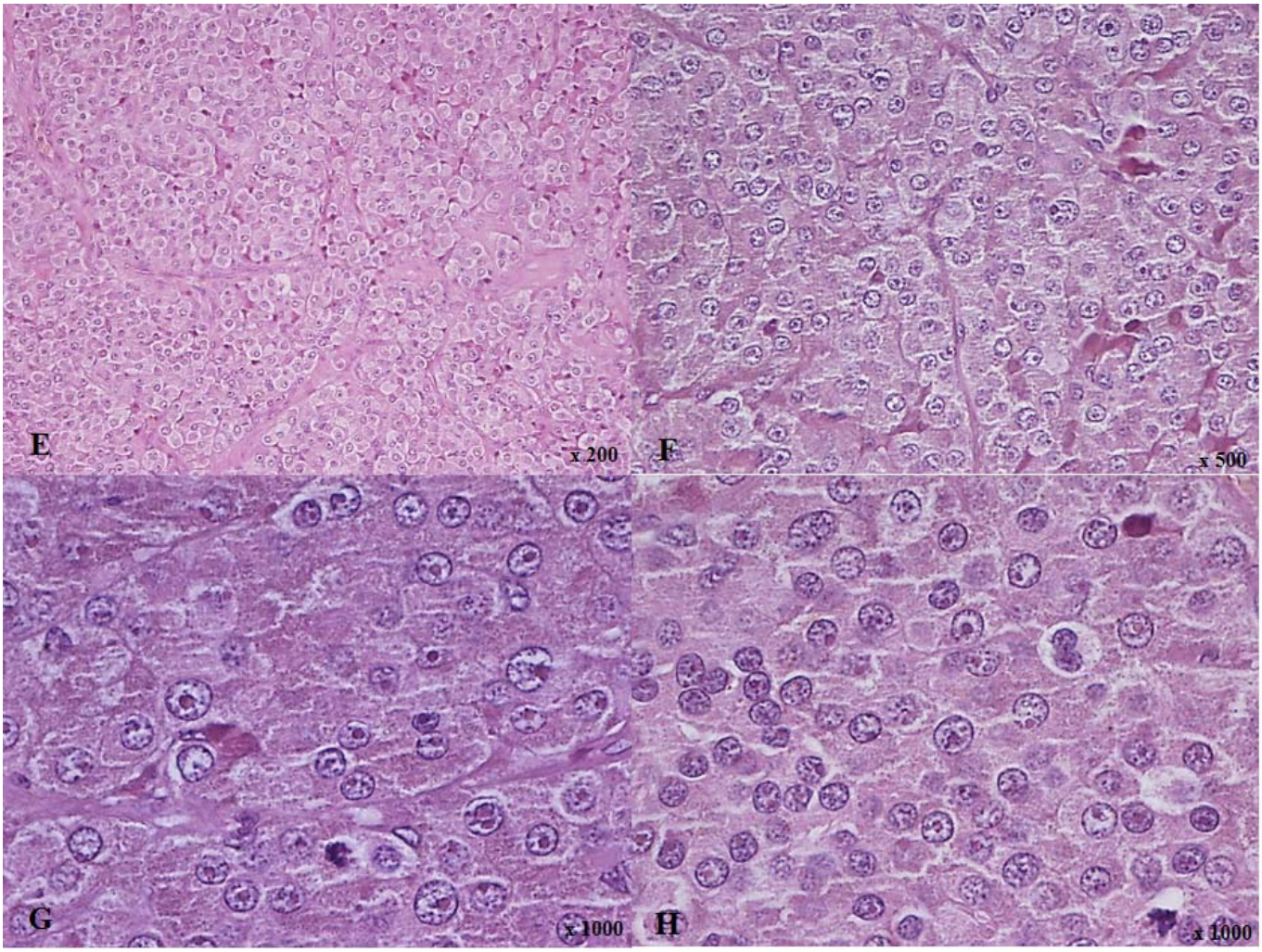
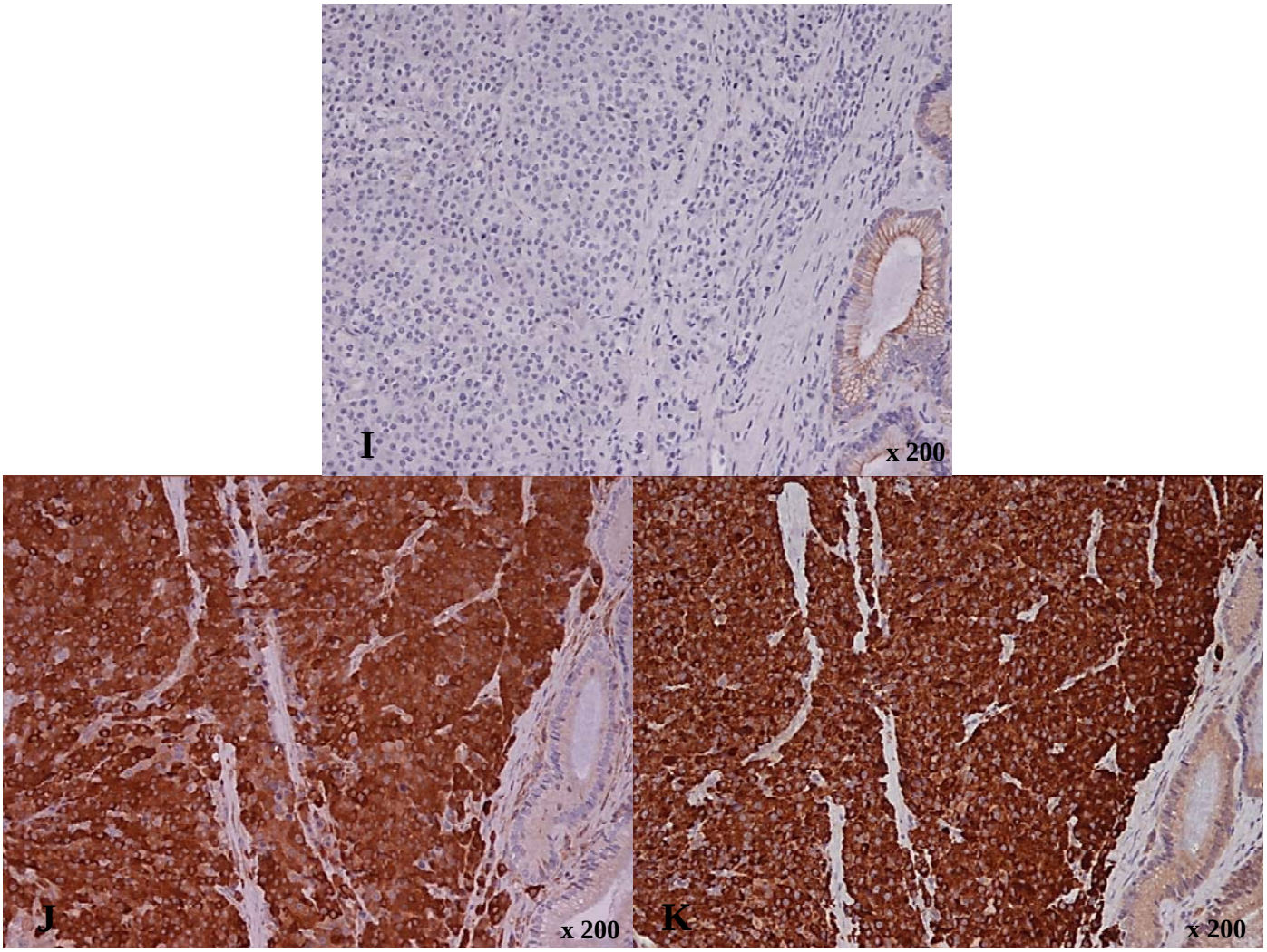


Figure 5: Immunohistochemistry performed on the biopsy of the gallbladder's mass. **I**: Cytokeratin (combination of cytokeratin 5, 6, 8, 17 and 19) negative staining of neoplastic cells (positive control: epithelial cells of the gallbladder on the right corner). **J**: Chromogranin A strongly positive staining of neoplastic cells (negative control: epithelial cells of the gallbladder on the right corner). **K**: Neuron Specific Enolase (NSE) strongly positive staining of neoplastic cells (negative control: epithelial cells of the gallbladder on the right corner). The immunohistochemistry panel is also consistent with a neuroendocrine tumor such as a carcinoid tumor.



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