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Carcinosarcoma of the Gallbladder Producing α-Fetoprotein and Manifesting as Leukocytosis with Elevated Serum Granulocyte Colony-Stimulating Factor: Report of a Case

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Abstract
A 69-year-old man was referred to our hospital for investigation of leukocytosis and a persistent fever of 38°C, but we could find no evidence of a specific infection. The leukocyte count was 18000/mm^3, and the serum granulocyte colony-stimulating factor (G-CSF) and α-fetoprotein (AFP) levels were both elevated, at 66.3 pg/ml and 1,495 ng/ml, respectively. Computed tomography (CT) showed a gallbladder tumor and we performed extended cholecystectomy. Postoperatively, the fever subsided and the leukocyte count, serum G-CSF and AFP level normalized. Histologically, the tumor was a carcinosarcoma of the gallbladder. Immunohistochemical staining of the tumor cells was positive for AFP, but negative for G-CSF. This is the first report of a carcinosarcoma of the gallbladder producing AFP. The laboratory findings and clinical course strongly suggested that the tumor produced not only AFP, but also G-CSF.

Key words Carcinosarcoma · Granulocyte colony-stimulating factor · α-Fetoprotein · Leukocytosis · Gallbladder

Introduction

Carcinosarcoma is a rare tumor, characterized by both malignant epithelial and mesenchymal components. This tumor arises most commonly in the gynecological or otorhinolaryngological regions, including the uterus, thyroid, esophagus, and upper respiratory tract.[1,2] Its most common origin in the gastrointestinal tract is the esophagus. Carcinosarcoma of the gallbladder is extremely rare, and there have been very few reports of this tumor producing granulocyte colony-stimulating factor (G-CSF) or α-fetoprotein (AFP). We report a case of carcinosarcoma of the gallbladder producing AFP, manifesting as leukocytosis and an elevated serum G-CSF level.

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Case Report

A 69-year-old man, complaining of right upper quadrant abdominal pain and fever, was referred to our hospital for investigation of leukocytosis of unknown origin. Physical examination revealed a temperature of 38.2°C and tenderness in the right upper quadrant of the abdomen. No lymphadenopathy or hepatosplenomegaly was found. Laboratory data showed anemia, leukocytosis (18000/mm^3), and a C-reactive protein (CRP) level of 18.46 mg/dl. The leukocytes consisted of 80% segmented forms, 6% eosinophils, 4% monocytes, and 10% lymphocytes. The serum concentrations of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were slightly elevated to 45 IU/l (normal range, 3–33 IU/l) and 466 IU/l (normal range, 126–336 IU/l), respectively. The tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9, were within the normal range, but the α-fetoprotein (AFP) level was high, at 1495 ng/ml (normal range, <10 ng/ml). Computed tomography (CT) showed an enhanced solid mass lesion, 6.5 cm × 6.5 cm × 6 cm, in the gallbladder (Fig. 1a). Abdominal ultrasonography (US) showed enlargement of the gallbladder and a heterogeneously echogenic mass occupying the entire gallbladder lumen, which was found by Doppler analysis to have a rich bloodstream (Fig. 1b). Magnetic resonance cholangiopancreatography (MRCP) showed choledocholithiasis, but the gallbladder was not described. The diagnosed choledocholithiasis was removed by therapeutic endoscopic retrograde cholangiography (ERCP). Gallium scintigraphy showed a high accumulation in the gall-

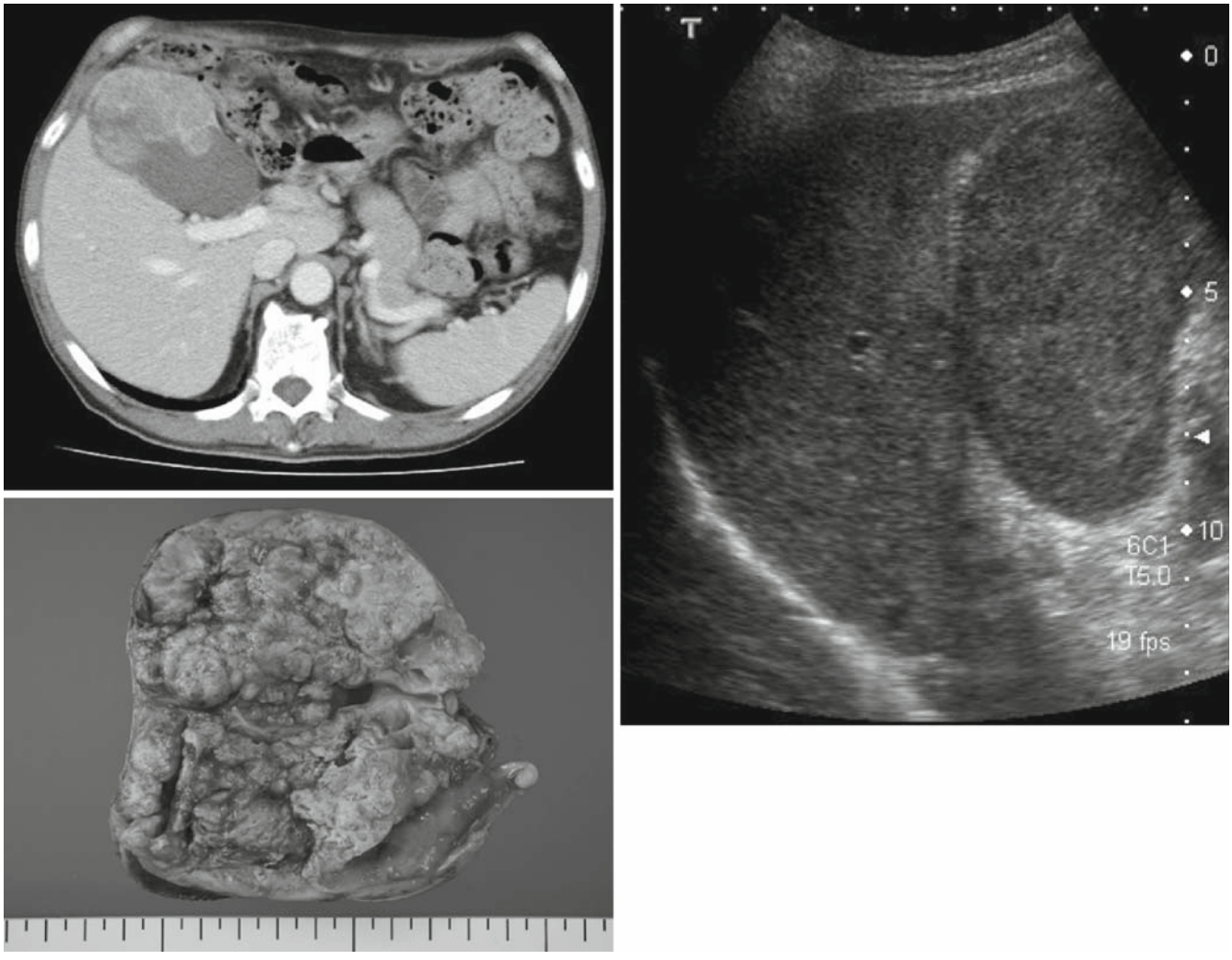

Fig. 1a–c. Preoperative examination and macroscopic findings of the resected specimen. **a** Computed tomography (CT) scan of the abdomen showed an enhanced solid mass, 6.5 cm × 6.5 cm × 6 cm, in the gallbladder. **b** Abdominal ultrasonography (US) showed enlargement of the gallbladder and a heterogeneously echogenic mass occupying the entire gallbladder lumen. **c** The cut surface of the resected specimen revealed a large yellowish-white solid tumor with necrotic changes occupying the entire lumen of the gallbladder

bladder. Extensive evaluation revealed no evidence of a specific infection or leukemia, although the patient's fever remained over 38°C and the leukocytosis did not disappear, despite several antibiotics. The maximal leukocyte count in the peripheral blood was 20300/mm^3. The serum G-CSF level, measured to find out if a G-CSF producing tumor was the underlying cause of the persistent leukocytosis, was elevated at 66.3 pg/ml (normal range, <30 pg/ml). We performed a laparotomy for a provisional diagnosis of carcinoma of the gallbladder producing G-CSF and AFP. No peritoneal dissemination of the tumor or swelling of the regional lymph nodes was found, so we performed cholecystectomy, resection of the liver bed, and lymph node dissection as a radical operation. The resected specimen contained a tumor measuring 9 cm × 5 cm. A cross section revealed a large yellowish-white solid mass with necrotic changes, occupying the entire lumen of the gallbladder (Fig. 1c). Histologically, the tumor was made up of two different components: poorly differentiated tubular adenocarcinoma, and sarcomatous tumor cells with a pleomorphic or spindle shape (Fig. 2a). The sarcomatous component was almost completely located in the tumor, whereas the adenocarcinoma cells were only partially observed. Morphologically, a gradual transition was observed between these two components. Immunohistochemically, some of the sarcomatous cells were positive for cytokeratin (Dako, Kyoto, Japan), an epithelial marker (Fig. 2b). Moreover, the sarcomatous element was positive for vimentin (Dako), an interstitial marker, but

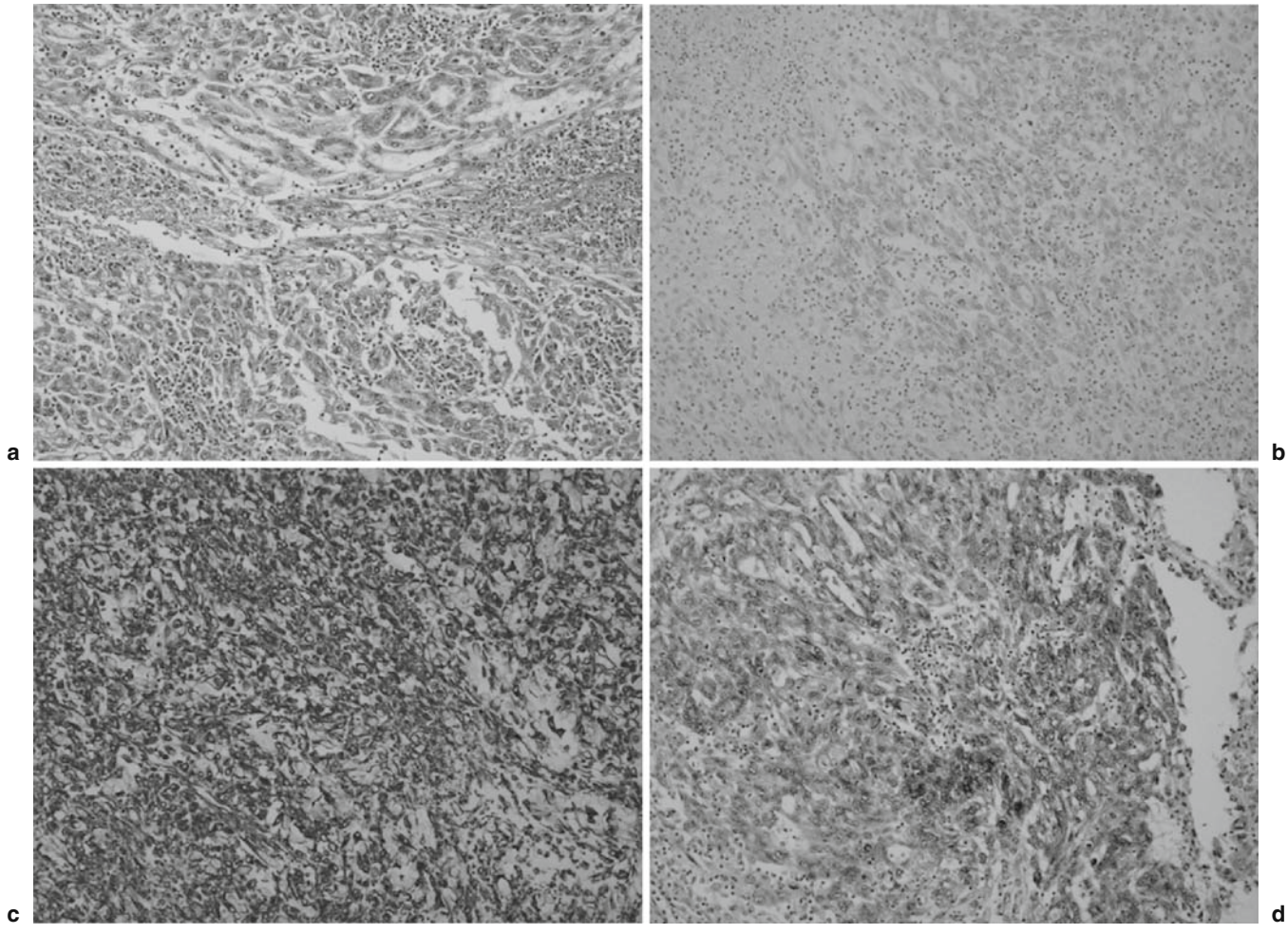

Fig. 2a–d. Microscopic findings of the resected specimen. **a** The tumor was composed of two different components: a poorly differentiated tubular adenocarcinoma and sarcomatous tumor cells with a pleomorphic or spindle shape. Morphologically, a gradual transition was observed between these two components (H&E staining, ×100). **b** Immunohistochemical staining revealed that some of the sarcomatous cells were positive for cytokeratin (×100). **c** The sarcomatous element was also positive for vimentin (×100). **d** Further immunohistochemical examination revealed that the tumor cells were positive for α-fetoprotein (×100)

negative for α-SMA (Dako) and S-100 protein (Dako) (Fig. 2c). Based on these findings, we made a definitive diagnosis of "carcinosarcoma of the gallbladder with subserosal invasion, which had not metastasized to the lymph nodes." A further immunohistochemical examination using antibodies directed against AFP (Dako) and G-CSF (Immuno-Biological Laboratories, Gunma, Japan) revealed that the tumor cells were positive for AFP and negative for G-CSF (Fig. 2d). After the operation, the leukocyte count returned to normal and the fever subsided immediately. The serum G-CSF level decreased to a normal level of 28 pg/ml (normal range, <30 pg/ml) by 1 month postoperatively. The serum AFP level decreased to 17.8 ng/ml by 1 month, and then to 2.8 ng/ml (normal range, <10 ng/ml) by 2 months postoperatively. The patient has been well for 6 months with no evidence of recurrence.

Discussion

Carcinosarcoma is characterized by malignancy of both the epithelial and mesenchymal components within the same tissue. They are generally classified into two groups: "true carcinosarcomas" and "so-called carcinosarcomas."[3] "True carcinosarcomas," in which a carcinoma and sarcoma arise simultaneously, have apparent sarcomatous differentiation toward specific tissues such as osteoid, cartilage, or striated muscle. "So-called carcinosarcomas," in which a sarcomatous reaction occurs in a carcinoma, have epithelial differentiation in a sarcomatoid component, and are termed "sarcomatoid carcinoma" or "spindle cell carcinoma." In the present case, the epithelial and sarcomatous components transferred to each other partially, and epithelial differentiation was detected in the sarcomatous component by

light microscopy and immunocytochemistry. Moreover, the sarcomatous tumor cells lacked any specific sarcomatous differentiation, so the tumor was diagnosed as a "so-called carcinosarcoma." However, it seems that it is sometimes difficult to distinguish between "true carcinosarcoma" and "so-called carcinosarcoma," and the mechanism of carcinogenesis in the tumor is still unclear because of its rarity.

To our knowledge, since carcinosarcoma of the gallbladder was first described in 1907 by Landsteiner,[4] only 36 cases have been reported in the English literature. According to the report of Hotta et al.,[5] carcinosarcoma of the gallbladder occurred predominantly in women, with a mean age of 67 years (range 45–91 years). The female to male ratio was 4:1 and about 75% of the patients had gallstones simultaneously. The epidemiology was similar to the more common types of gallbladder cancer, but the prognosis of this disease was poorer, with a mean survival after diagnosis of only a few months.[5] To our knowledge, there has been no other report of an accurate preoperative diagnosis of carcinosarcoma of the gallbladder.

α-Fetoprotein is a clinically useful and reliable marker for the diagnosis of primary hepatocellular carcinoma, hepatoblastoma, and yolk-sac tumors. Elevated serum AFP levels have also been reported occasionally in patients with malignant tumors of the gastrointestinal tract, such as the stomach, pancreas, or duodenum, but an AFP-producing tumor originating in the gallbladder is rare. Our search of the world literature found no other report of a carcinosarcoma of the gallbladder with an elevated serum AFP level. This tumor was positive for AFP staining and the serum AFP level decreased to a normal level after its resection. Thus, this is the first reported case of a carcinosarcoma of the gallbladder producing AFP. Hayashi et al.[6] suggested that AFP-producing carcinomas of the gallbladder metastasize to the liver more frequently, and that the prognosis is poorer than that of non AFP-producing carcinomas of the gallbladder.

Granulocyte-CSF regulates the proliferation and differentiation of granulocytes, functioning as the hematopoietic growth factor responsible for the marked leukocytosis in G-CSF-producing tumors, as seen in paraneoplastic syndrome. Since the production of G-CSF by malignant cells was first identified in lung cancer in 1977,[7] it has been seen in various malignancies,[8] but primarily in lung cancer. Moreover, G-CSF-producing tumors often simultaneously produce cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-α.[9] Consequently, patients suffer general fatigue and a continuous fever because of the chronic inflammation. There are few reports of G-CSF-producing tumors originating in the gallbladder. The G-CSF-producing tumor is diagnosed on the basis of an elevated serum G-CSF level and immunohistochemical confirmation of the production of G-CSF in the tumor tissue. In the present case, an immunohistochemical study failed to show G-

Table 1. Clinicopathological characteristics of the reported cases of gallbladder tumors causing leukocytosis, which were diagnosed as or suspected of being G-CSF-producing tumors

Case	First author (year)[Ref.]	Age (years)	Sex	Symptoms	WBC	G-CSF	Operation	Histology	Prognosis
1	Nishimaki (1982)[11]	72	F	Pain, nausea	44100	ND	(–)	adenosq	1m, dead
2	Takahashi (1985)[12]	72	F	Pain, nausea	41100	ND	(–)	ad	1m, dead
3	Sakamoto (1986)[13]	55	F	Anorexia, fatigue	57900	ND	chole-PRL	undiff	6m, dead
4	Watanabe (1989)[14]	54	F	Pain, fever	38000	ND	(–)	undiff	1.5m, dead
5	Takeda (1990)[15]	79	F	Anorexia, fatigue	12600	ND	chole	undiff	6m, dead
6	Oguri (1991)[16]	58	M	Mass	131500	181	chole-PRL	undiff	3m, dead
7	Nakajima (1996)[17]	71	M	Fatigue, fever	22400	238	chole-PRL	adenosq	6m, dead
8	Omura (1999)[18]	73	M	Fever	75200	129	chole-LBR	undiff	18m, alive
9	Furihata (1999)[19]	48	F	Pain, fever	15700	54	chole-PRL	ad (por)	11m, alive
10	Yamakawa (1999)[20]	79	M	Pain	54700	235	(–)	ND	2m, dead
11	Kuroki (2000)[21]	73	F	Pain	35000	1311	(–)	ad (mod)	1m, dead
12	Murata (2001)[22]	62	M	Pain	46940	50.8	chole	sq (mod)	3m, dead
13	Nakamura (2001)[23]	68	M	Fever	40570	242	(–)	ad (por)	4m, dead
14	Hanashiro (2001)[24]	77	M	Pain, fever	18800	ND	chole	CS	1m, dead
15	Kato (2002)[25]	70	M	Mass	50700	133	chole-LBR	IMFH	36m, alive
16	Shizuma (2003)[26]	79	F	None	37000	122	(–)	ad (mod)	4m, dead
17	Yoshida (2004)[27]	78	F	Pain	26200	ND	chole-PRL	sq	12m, dead
18	Ikeda (2005)[28]	50	F	Pain	51500	800	chole-ERLL	ad (mod)	27m, alive
19	Our case	69	M	Pain, fever	18000	66.3	chole-LBR	CS	5m, alive

WBC, white blood cells; G-CSF, granulocyte colony-stimulating factor; ND, no data; chole, cholecystectomy; PRL, partial resection of the liver; LBR, liver bed resection; ERLL, extended right lobectomy of the liver; adenosq, adenosquamous carcinoma; ad, adenocarcinoma; undiff, undifferentiated carcinoma; sq, squamous cell carcinoma; por, poorly differentiated; mod, moderately differentiated; IMFH, inflammatory malignant fibrous histiocytoma; CS, carcinosarcoma; m, months

CSF production by the tumor cells, but the leukocyte count and serum G-CSF level normalized after surgery and the persistent high fever disappeared. These laboratory findings and the clinical course thus suggests that the carcinosarcoma of the gallbladder produced G-CSF. The tumor cells were not stained with the anti-G-CSF antibody because the intracellular retention of the G-CSF protein is very brief. The cellular dynamics produce a quick turnover, leaving only a small amount of the protein, which is difficult to detect.[10] In the world literature, we found only 19 cases of gallbladder tumors with leukocytosis, which were diagnosed as, or suspected of being G-CSF-producing tumors, including our case (Table 1).[11–28] It appeared that the serum G-CSF level did not correlate with the leukocyte count. Histopathologic examination revealed an undifferentiated carcinoma in five cases, adenosquamous carcinoma in two cases, and carcinosarcoma in two cases. These pleomorphic-type carcinomas are very rare, but they may be characteristic of G-CSF-producing tumors of the gallbladder.

Granulocyte-CSF-producing tumors are generally associated with a poor prognosis because of their rapid progression and metastasis. The reported survival period after diagnosis is about 3 months, indicating that it is very aggressive.[28] Eight of the patients reported died within 3 months, and four of the nine patients who underwent curative resection died within 12 months (Table 1). Despite the very poor prognosis, curative resection provides the only chance of long-term survival, so a radical operation should be performed early. Delaying the operation results not only in deterioration in the patient's condition because of the progressive inflammation, but it may prevent any chance of survival. We ruled out an infectious disease or leukemia immediately in our patient and operated 25 days after his admission, which led to the decision to perform radical surgery. We suspected the production of both G-CSF and AFP by the tumor cells. Measuring the serum G-CSF and AFP levels is particularly useful for detecting a metastatic recurrence early because of their close correlation with tumor growth. Although we have found no sign of recurrence in our patient after 6 months, he must be followed up very carefully in view of the aggressive biological behavior of this tumor.

References

1. Jonson AL, Bliss RL, Truskinovsky A, Judson P, Argenta P, Carson L, et al. Clinical features and outcomes of uterine and ovarian carcinosarcoma. Gynecol Oncol 2006;100:561–4.
2. Batsakis JG, Suarez P. Sarcomatoid carcinomas of the upper aerodigestive tracts. Adv Anat Pathol 2000;7:282–93.
3. Humphrey PA, Scroggs MW, Roggli VL, Shelburne JD. Pulmonary carcinomas with a sarcomatoid element: an immunocytochemical and ultrastructural analysis. Hum Pathol 1988;19:155–65.
4. Landsteiner K. Plattenepithelkarzinom und sarkom der gallenblase in einem falle von cholelithiasis. Ztschr Klin Med 1907; 62:427–33.
5. Hotta T, Tanimura H, Yokoyama S, Ura K, Yamaue H. So-called carcinosarcoma of the gallbladder; spindle cell carcinoma of the gallbladder: report of a case. Surg Today 2002;32:462–7.
6. Hayashi K, Hiraki M, Yamashita Y, Kurohiji T, Kimitsuki H, Watanabe J, et al. A case of cancer of the gallbladder with a high level of alphafetoprotein (in Japanese with English abstract). Nippon Rinsyo Geka Gakkai Zasshi (J Jpn Soc Clin Surg) 1994;55:3161–5.
7. Asano S, Urabe A, Okabe T, Sato N, Kondo Y. Demonstration of granulopoietic factor(s) in the plasma of nude mice transplanted with a human lung cancer and in the tumor tissue. Blood 1977;49:845–52.
8. Fujita T, Ogasawara Y, Naito M, Doihara H, Shimizu N. Anaplastic thyroid carcinoma associated with granulocyte colony-stimulating factor: report of a case. Surg Today 2006;36: 63–7.
9. Asakawa H, Kobayashi T. The secretion of cytokines and granulocyte colony-stimulating factor by anaplastic and poorly differentiated thyroid carcinoma cell lines. Anticancer Res 1999;19: 761–4.
10. Shimamura K, Fujimoto J, Hata J, Akatsuka A, Ueyama Y, Watanabe T, et al. Establishment of specific monoclonal antibodies against recombinant human granulocyte colony-stimulating factor (hG-CSF) and their application for immunoperoxidase staining of paraffin-embedded sections. J Histochem Cytochem 1990;38:283–6.
11. Nishimaki T, Yoshida K, Takakuwa K, Hirota M, Muto T, Takahashi M, et al. A case of carcinoma of the gallbladder producing colony-stimulating factor (CSF) (in Japanese). Nippon Shokakibyo Gakkai Zasshi 1982;79:1336–40.
12. Takahashi M, Fujiwara M, Kishi K, Sakai C, Sanada M, Moriyama Y, et al. CSF producing gallbladder cancer: case report and characteristics of the CSF produced by tumor cells. Int J Cell Cloning 1985;3:294–303.
13. Sakamoto K, Egami H, Yoshimura R, Nakamura S, Ikei S, Mori K, et al. Colony-stimulating factor producing carcinoma of the gallbladder. Jpn J Clin Oncol 1986;16:87–96.
14. Watanabe Y, Ogino Y, Ubukata E, Sakamoto Y, Matsuzaki O, Shimizu N. A case of a gallbladder cancer with marked hypercalcemia and leukocytosis. Jpn J Med 1989;28:722–6.
15. Takeda T, Ichiyanagi A, Sano K, Yoshida J, Tsutsumi Y, Miyaji T. A case of gallbladder cancer producing granulocyte colony-stimulating factor. Gastroenterol Jpn 1990;25:762–7.
16. Oguri T, Ikemoto H, Sato H, Abe M, Narahara M, Koyanagi T, et al. A case of gallbladder cancer producing colony stimulating factor with rapid progression (in Japanese). Oitaken Igakukai Zasshi 1991;10:81–4.
17. Nakajima Y, Takashima T, Naito E, Yoshida J, Senmaru H, Oka M, et al. Case of G-CSF producing gallbladder neoplasm (in Japanese). Nippon Naika Gakkai Zasshi 1996;85:1931–3.
18. Omura N, Abe S, Hirai K, Aoki T. A case of granulocyte colony-stimulating factor producing gallbladder cancer. Am J Gastroenterol 1999;94:273–5.
19. Furihata M, Sonobe H, Ohtsuki Y, Enzan H, Tokuoka H, Nakanuma Y. An immunohistochemical study on a case of granulocyte-colony stimulating factor-producing gall-bladder carcinoma. Pathol Int 1999;49:1010–3.
20. Yamakawa M, Mizuta Y, Mori I, Doi M, Isomoto H, Takeshima F, et al. A case of gallbladder cancer presumed to have produced granulocyte-colony stimulating factor (in Japanese). Rinsyo to Kenkyu 1999;76:1779–82.
21. Kuroki M, Uto H, Ido A, Kuwata G, Nakama T, Ochiai T, et al. A case of gallbladder cancer producing granulocyte-colony stimulating factor and possible parathyroid hormone related protein

(in Japanese). Nippon Shokakibyo Gakkai Zasshi 2000;97:478–83.
22. Murata M, Tateishi H, Nishiyama H, Ito M, Zushi S, Imai Y, et al. A case of granulocyte-colony stimulating factor producing squamous cell carcinoma of the gallbladder (in Japanese). Nippon Shokakibyo Gakkai Zasshi 2001;98:53–7.
23. Nakamura M, Nishikawa S, Onozawa M, Takagi K, Abo D, Kudoh T, et al. A case of gallbladder cancer producing granulocyte-colony stimulating factor (in Japanese with English abstract). Shiritsu Sapporo Byoin Ishi 2001;61:3–8.
24. Hanashiro N, Tamaki S, Naka T, Muto Y. Carcinosarcoma (sarcomatoid carcinoma) of the gallbladder presenting with a cholecystocolic fistula and a marked leukocytosis: a case report. Ryukyu Med J 2001;20:77–80.
25. Kato T, Kojima T, Shimizu T, Sasaki H, Abe M, Okushiba S, et al. Inflammatory malignant fibrous histiocytoma of the gallbladder: report of a case. Surg Today 2002;32:81–5.
26. Shizuma T, Obata H, Hashimoto E, Ikeda I. A case of gallbladder carcinoma accompanied with high levels of serum granulocyte colony-stimulating factor and alpha-fetoprotein (in Japanese with English abstract). Tokyo Joshi Ika Daigaku Zasshi 2003;73:194–9.
27. Yoshida M, Tabo T, Hayashi H, Onodera H, Imamura Y. A case of squamous cell carcinoma of the gallbladder producing granulocyte-colony stimulating factor (in Japanese with English abstract). Nippon Rinsyo Geka Gakkai Zasshi (J Jpn Soc Clin Surg) 2004;65:2459–63.
28. Ikeda T, Ohgaki K, Miura M, Aishima S, Shimizu T, Maehara Y. Granulocyte-colony stimulating factor-producing gallbladder cancer without recurrence more than 2 years after resection: report of a case. Surg Today 2005;35:590–3.