INTERNAL MEDICINE

□ CASE REPORT □

Granulocyte-Colony Stimulating Factor-Producing Pancreatic Adenosquamous Carcinoma Showing Aggressive Clinical Course

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Abstract

Herein, we encountered an 89-year-old woman with pancreatic cancer who presented with fever without infective focus, leukocytosis of 45,860/μL, and elevation of serum granulocyte-colony stimulating factor (G-CSF). The patient could not receive any curative therapy due to an extremely aggressive clinical course. Specimens taken at necropsy revealed an adenosquamous carcinoma positive for G-CSF by immunohistochemistry; it was only the second reported case to date. She was finally diagnosed with G-CSF-producing pancreatic cancer. In light of the above, clinicians should consider the presence of G-CSF-producing tumors, including pancreatic cancer, when presented with patients showing leukocytosis of unknown origin and fever without infective focus.

Key words: granulocyte-colony stimulating factor, granulocyte-colony stimulating factor-producing cancer, pancreatic cancer, gastric cancer, immunohistochemistry, interleukin 6

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Introduction

The concept of colony stimulating factor (CSF) as a hematopoietic induction, differentiation, and growth factor was first discussed in 1966 (1). A case of malignancy was then later reported with increased CSF activation in serum and urine in 1974 (2). Afterwards, it was demonstrated for the first time that CSF was directly produced by a lung cancer tissue specimen in 1977 (3).

Granulocyte-colony stimulating factor (G-CSF) is recognized as a naturally occurring glycoprotein that stimulates the proliferation and maturation of precursor cells in the bone marrow into fully differentiated neutrophils (4, 5). Although several accounts of G-CSF-producing malignant tumors in lung cancer exist, few have been observed in the digestive system. Notably, there have been very few cases found in pancreatic cancer; the 8 previous reports concerning G-CSF-producing pancreatic cancer are summarized in Table 1 (6-12).

Herein, we present only the second case of G-CSF-producing pancreatic adenosquamous carcinoma, which we were able to confirm by immunohistochemistry.

Case Report

An 89-year-old woman had been admitted to another hospital three months prior to ours for rehabilitation of a compression fracture of the lumbar vertebra. As she suffered from anorexia, low grade fever, and weight loss and was

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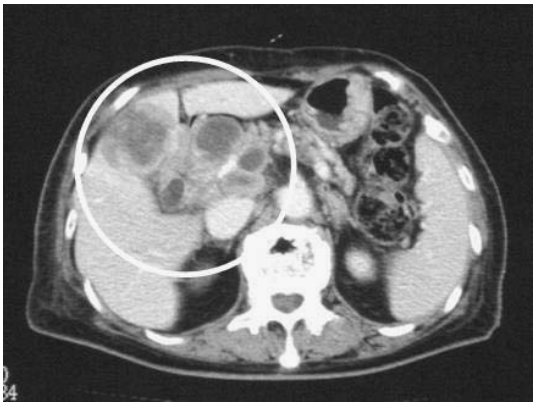

Figure 1. **Enhanced abdominal computed tomography showed a part of the tumor was located from the pancreatic head and uncus to the porta hepatica (white circle). Multiple metastatic lesions in the liver parenchyma were simultaneously noticed (not shown).**

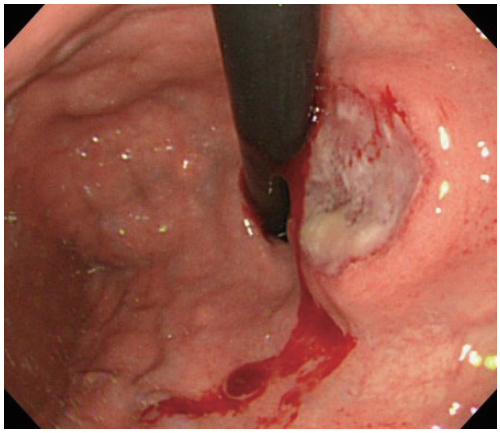

Figure 2. **A Borrmann type 2 gastric cancer was detected by esophageal gastric duodenoscopy.**

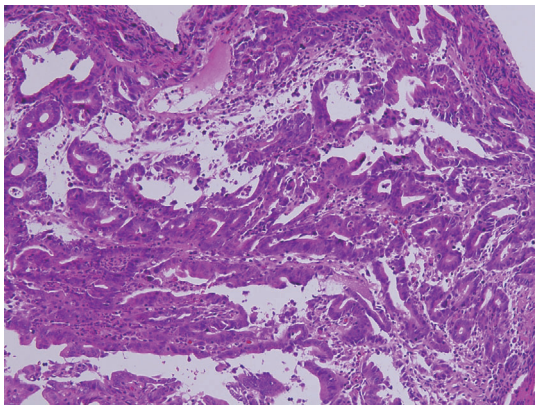

Figure 3. **A specimen taken by stomach biopsy revealed atypical epithelium showing papillary proliferation and was considered to be a well-differentiated adenocarcinoma. Immunohistochemical staining for granulocyte-colony stimulating factor (G-CSF) was negative (not shown).**

found to have leukocytosis of 53,400/μL, she was transferred to our hospital for further examination. Lung cancer, gastric cancer, and prostatic cancer were noted among her family members.

On examination, the patient was 147.5 cm tall and weighed 46.1 kg. Her body temperature was 36.5℃. She appeared well with neither signs of anemia nor jaundice in conjunctiva. Neurological, chest, and other abdominal examinations revealed no abnormal findings.

Blood tests showed a white blood cell count of 45,860/μL with 96% neutrophils, a red blood cell count of 369×10^4/μL, and a platelet count of 46.5×10^4/μL. The patient's hemoglobin value was 10.8 g/dL and her hematocrit was 33.4%. Blood chemistry showed a serum albumin of 3.2 mg/dL, alkaline phosphatase of 822 U/L, gamma-glutamyl transpeptidase of 130 U/L, total bilirubin of 0.5 mg/dL, and creatinine of 0.8 mg/dL. Serum transaminase was within normal limits, and blood serology showed a C-reactive protein (CRP) of 11.98 mg/dL. Serum carbohydrate antigen 19-9 (CA19-9) was elevated at 959 U/mL. Levels of carcinoembryonic antigen (CEA) and α-fetoprotein (AFP) were within normal limits at 2.5 ng/mL and 5.0 ng/mL, respectively.

The patient's most prominent clinical feature was severe leukocytosis, but chronic myelogenous leukemia (CML) was ruled out since peripheral blood analysis showed no immature cells and her neutrophil alkaline phosphatase (NAP) score was 476 (normal value: 150-330). A tumor mass was noted from the pancreatic head and uncus to the porta hepatica with multiple metastatic lesions in the liver in abdominal computed tomography (CT) (Fig. 1) and ultrasonography (US). Fever without chills was observed every evening after admission, which was considered to be related to tumor growth. Supplemental oral nutrients were provided immediately due to her poor dietary intake. Advanced gastric cancer of Borrmann type 2 was detected in esophageal gastric duodenoscopy (Fig. 2) with histological evidence of a well-differentiated adenocarcinoma (Fig. 3). Serum elevation of G-CSF at 690 pg/mL (normal value: less than 18.1 pg/mL) and interleukin-6 (IL-6) at 33.8 pg/mL (normal value: less than 4.0 pg/mL) were noted. Serum values of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) were within normal limits. Jaundice gradually appeared with elevation of serum hepatobiliary enzyme. Endoscopic retrograde cholangiopancreatography (ERCP) showed irregular narrowing of the common bile duct from the porta hepatica to the lower bile duct (Fig. 4), which suggested pancreatic head cancer and direct invasion into the bile duct and lymph nodes. An endoscopic retrograde bile duct drainage tube was inserted to relieve the obstructive jaundice. Brush cytology of the bile duct showed a Class II and a histological biopsy revealed a Group I with no malignancy.

Multiple new metastatic lesions in the lungs began appearing in chest X-rays and CT on day 20 after admission. As disease progression was alarmingly fast, only palliative care was recommended. The patient's white blood cell count was 34,000/μL on day 22 and 140,570/μL on day 53. Dyspnea due to massive pleural effusion and abdominal full-

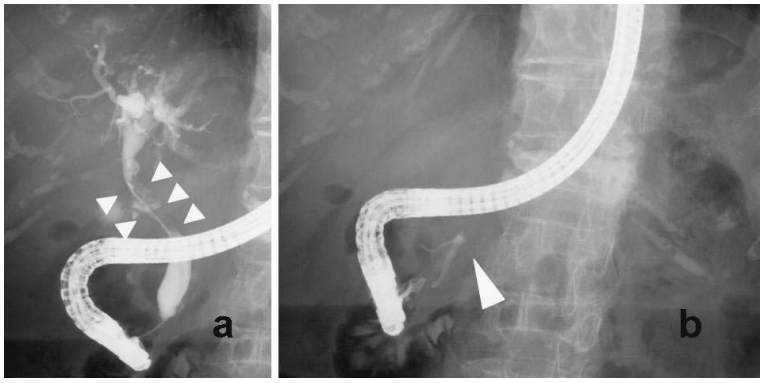

Figure 4. **a) Irregular narrowing of the common bile duct induced by extrinsic pressure was seen (arrowheads) from the porta hepatica to the lower bile duct by endoscopic retrograde cholangiography. b) Endoscopic retrograde pancreatography showed obstruction of the main pancreatic duct (arrowhead).**

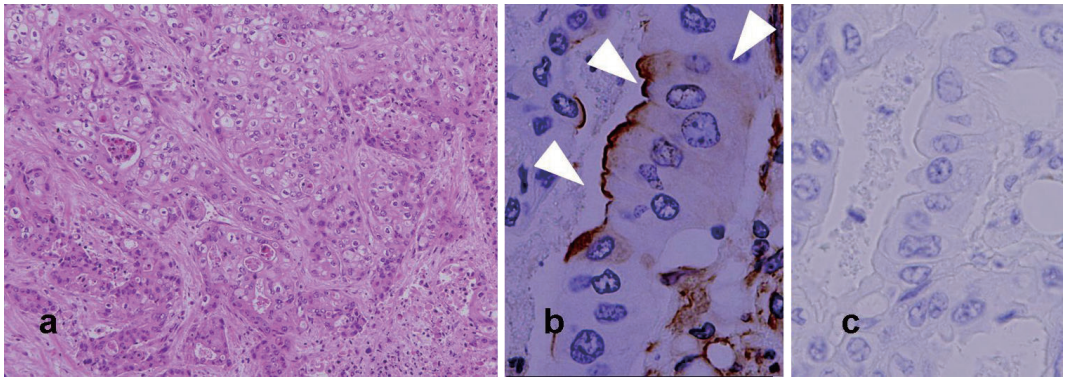

Figure 5. **a) Microscopic findings showed atypical cells lying in sheets and composed of a poorly differentiated adenosquamous carcinoma. Hematoxylin and Eosin staining, ×10 original magnification. b) Immunohistochemical examination showed positive staining for granulocyte-colony stimulating factor (G-CSF) in the cytoplasm and surface of the atypical cells (arrowheads). ×40 original magnification. c) Negative control. ×40 original magnification.**

ness from massive ascites appeared, and she passed away on day 62. We later conducted a necropsy with informed consent from her family. The specimens taken from an ultrasound-guided tumor biopsy showed a poorly-differentiated adenosquamous carcinoma with positive staining for G-CSF by immunohistochemistry (anti-G-CSF antibody, Calbiochem Ab-1, Darmstadt) (Fig. 5). Tissue specimens from concomitant gastric cancer were negative for G-CSF staining. We also tested for G-CSF receptor by immunostaining (anti-G-CSF receptor antibody, Abcam S-1284, Cambridge) but found no positive findings. Based on these results, she was diagnosed with G-CSF-producing pancreatic cancer.

Discussion

The immunological findings in our case are strongly indicative that the patient's pancreatic cancer produced G-CSF. Clinicians sometimes encounter leukocytosis of unknown origin in patients with advanced stage malignancies, and a number of these have been reported to be associated with a G-CSF-producing tumor. The diagnostic criteria for such a tumor are the following: 1) extreme leukocytosis, 2) elevated G-CSF activity, 3) fall-off of white blood cell count after tumor resection, or 4) proof of G-CSF production in the tumor (3). Both extreme leukocytosis and significant elevation of serum G-CSF were noticed in this patient. A reliable way to prove tumoral G-CSF production is by immunostaining (13). In our case, we directly proved by immunochemistry of pancreatic cancer tissue specimens taken at necropsy that the tumor had produced G-CSF. Since specimens taken from her concomitant gastric tumor showed the different tumor type of well-differentiated adenocarcinoma that was negative for G-CSF, we could confidently diagnose this patient with G-CSF-producing pancreatic cancer.

Most patients with pancreatic cancer experience pain, weight loss, or jaundice (14, 15). Notably, pain is present in 80 to 85 percent of patients with locally advanced or advanced disease (14). In addition, half of the 8 published cases of G-CSF-producing pancreatic cancer showed continuous fever without infective focus seen in this patient (Table 1). Accordingly, such a symptom may be a clinical sign

Table 1. Published Cases of Granulocyte-colony Stimulating Factor-producing Pancreatic Cancer

Case	Age (yrs.)	Gender	Symptoms	Location	WBC (/μL)	Neut. (%)	G-CSF (pg/mL) (normal value)	CRP (mg/dL)	Pathological diagnosis	Therapies	Prognosis
Case 1 [6]	72	W	ND	ND	61,000	91	86 (<60)	ND	ND	ND	ND
Case 2 [7]	64	M	fever	Pt	24,300	93	157 (<30)	0.62	anaplastic carcinoma	operation	8 weeks after operation
Case 3 [8]	83	M	back pain	Pb	15,700	81	123 (6.0~21.9)	ND	poorly differentiated adenosquamous carcinoma	chemotheraphy	120 hospital days
Case 4 [9]	60	M	weight loss, fever	Pt	28,900	70	77 (<30)	15.16	poorly differentiated adenocarcinoma	palliative care	46 hospital days
Case 5 [10]	50	M	leukocytosis	ND	49,180	ND	350 (5~15)	ND	poorly differentiated adenocarcinoma	palliative care	20 hospital days
Case 6 [11]	46	M	general fatigue	Pbt	14,300	84	155 (5.78~27.5)	4.6	anaplastic carcinoma	operation	4months after operation
Case 7 [12]	74	M	fever	Pbt	29,500	ND	110 (<18.1)	15.9	poorly differentiated adenocarcinoma	operation	42 days after operation
Our case	89	W	weight loss, fever	Ph	45,860	96	690 (<18.1)	11.98	poorly differentiated adenosquamous carcinoma	palliative care	62 hospital days

Abbreviations: M: man, W: Woman, ND: not described, WBC: white blood cells, Neut: neutrophils, G-CSF: granulocyte-colony stimulating factor, CRP: C-reactive protein, Ph: pancreatic head, Pb: pancreatic body, Pbt: pancreatic body and tail, Pt: pancreatic tail

of G-CSF-producing pancreatic cancer. IL-6 overproduction and high levels of serum CRP were also noticed in the present case, which may have been responsible for the fever. In a previous report, co-production of G-CSF and IL-6 was associated with IL-1 production in G-CSF-producing cancer cell lines (15). IL-6 is considered to act as an endogenous pyrogen (16, 17) that regulates the synthesis of acute phase proteins, including CRP (18, 19).

The present case showed an aggressive clinical course of 2 months with new metastatic lung lesions appearing on day 20 of admission, which may have been related to G-CSF overproduction from the pancreatic tumor. Pancreatic cancer is in itself an extremely aggressive malignant tumor characterized by extensive invasion and very early metastasis (20). The median survival rates in patients undergoing radical resection for this disease are from 11.5 to 26 months (21, 22), and the median survival time for patients receiving gemcitabine chemotherapy is 5.65 months (23). In addition, the prognosis of G-CSF-producing tumors is generally considered to be poor (24), especially in cases with G-CSF-producing pancreatic cancer because all cases reported thus far died within 4 months of detection, regardless of therapeutic course (Table 1). It has been demonstrated that G-CSF stimulated the growth of a non-hematopoietic malignant cell line *in vivo* (25), and G-CSF was also considered to be a major autocrine growth factor in rapid tumor proliferation and metastasis (26, 27). We were unable to prove the presence of G-CSF receptors in the tumor by immunostaining in this case; it is possible that the antibody used was not sensitive enough for this tumor.

In conclusion, clinicians should consider G-CSF-producing pancreatic cancer when encountering patients with pancreatic cancer who show leukocytosis of unknown origin and fever without infective focus. In addition, pancreatic cancer with G-CSF production may have an exceptionally aggressive clinical course compared with that without G-CSF production.

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References

1. Bradley TR, Metcalf D. The growth of mouse bone marrow cells in vitro. Aust J Exp Biol Med Sci **44**: 287-299, 1966.
2. Robinson WA. Granulocytosis in neoplasia. Ann N Y Acad Sci **230**: 212-218, 1974.
3. Asano S, Urabe A, Okabe T, et al. Demonstration of granulopoietic factor(s) in the plasma of nude mice transplanted with a human lung cancer and in the tumor tissue. Blood **49**: 845-852, 1977.
4. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (2). N Engl J Med **327**: 99-106, 1992.
5. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (1). N Engl J Med **327**: 28-35, 1992.
6. Nakamura K, Takahashi T, Tsuyuoka R, et al. Identification of colony-stimulating factor activity in patients with malignant tumors associated with excessive leukocytosis. Jpn J Clin Oncol **21**: 395-399, 1991.
7. Uematsu T, Tsuchie K, Ukai K, et al. Granulocyte-colony stimulating factor produced by pancreatic carcinoma. Int J Pancreatol **19**: 135-139, 1996.
8. Ohtsubo K, Mouri H, Sakai J, et al. Pancreatic cancer associated with granulocyte-colony stimulating factor production confirmed by immunohistochemistry. J Clin Gastroenterol **27**: 357-360, 1998.
9. Yokoi H, Nakata M, Sawai K, et al. Intraglomerular metastasis from pancreatic cancer. Am J Kidney Dis **37**: 1299-1303, 2001.
10. Fukushima N, Sasatomi E, Tokunaga O, et al. A case of pancreatic cancer with production of granulocyte colony-stimulating factor. Am J Gastroenterol **96**: 258-259, 2001.
11. Gotohda N, Nakagohri T, Saito N, et al. A case of anaplastic ductal carcinoma of the pancreas with production of granulocyte-

colony stimulating factor. Hepatogastroenterology **53**: 957-959, 2006.
12. Takami K, Miura K, Takeuchi H, et al. Granulocyte-colony stimulating factor-producing pancreatic cancer: report of a case. Surg Today **38**: 453-457, 2008.
13. Shimamura K, Fujimoto J, Hata J, 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 **38**: 283-286, 1990.
14. Kalser MH, Barkin J, MacIntyre JM. Pancreatic cancer. Assessment of prognosis by clinical presentation. Cancer **56**: 397-402, 1985.
15. Suzuki A, Takahashi T, Okuno Y, et al. IL-1 production as a regulator of G-CSF and IL-6 production in CSF-producing cell lines. Br J Cancer **65**: 515-518, 1992.
16. Dinarello CA. Cytokines as endogenous pyrogens. J Infect Dis **179** Suppl 2 : S294-304, 1999.
17. Luheshi GN. Cytokines and fever. Mechanisms and sites of action. Ann N Y Acad Sci **856**: 83-89, 1998.
18. Castell JV, Gomez-Lechon MJ, David M, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett **242**: 237-239, 1989.
19. Castell JV, Gomez-Lechon MJ, David M, et al. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. Hepatology **12**: 1179-1186, 1990.
20. Keleg S, Buchler P, Ludwig R, et al. Invasion and metastasis in pancreatic cancer. Mol Cancer **2**: 14, 2003.
21. Farnell MB, Pearson RK, Sarr MG, et al. A prospective randomized trial comparing standard pancreatoduodenectomy with pancreatoduodenectomy with extended lymphadenectomy in resectable pancreatic head adenocarcinoma. Surgery **138**: 618-628; discussion 628-630, 2005.
22. Yeo CJ, Cameron JL, Lillemoe KD, et al. Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma, part 2: randomized controlled trial evaluating survival, morbidity, and mortality. Ann Surg **236**: 355-366; discussion 366-358, 2002.
23. Burris HA 3rd, Moore MJ, Andersen J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol **15**: 2403-2413, 1997.
24. Higaki I, Hirohashi K, Fukushima S, et al. Renal pelvic carcinoma producing granulocyte colony-stimulating factor: report of a case. Surg Today **31**: 266-268, 2001.
25. Segawa K, Ueno Y, Kataoka T. In vivo tumor growth enhancement by granulocyte colony-stimulating factor. Jpn J Cancer Res **82**: 440-447, 1991.
26. Tachibana M, Miyakawa A, Tazaki H, et al. Autocrine growth of transitional cell carcinoma of the bladder induced by granulocyte-colony stimulating factor. Cancer Res **55**: 3438-3443, 1995.
27. Baba M, Hasegawa H, Nakayabu M, et al. Establishment and characteristics of a gastric cancer cell line (HuGC-OOHIRA) producing high levels of G-CSF, GM-CSF, and IL-6: the presence of autocrine growth control by G-CSF. Am J Hematol **49**: 207-215, 1995.