Microsatellite Instability-high-positive Cancer of Unknown Primary Origin with a Long-term Survival after Surgery Alone

Kai Korekawa¹,², Atsushi Kunimitsu³,⁴ and Rento Morishima³

Abstract:
An 80-year-old woman was referred to our hospital for a thorough examination of enlarged lymph nodes on the lesser curvature of the stomach. Upon suspicion of malignant lymphoma, the patient underwent open lymphadenectomy and was diagnosed with lymph node metastasis of poorly differentiated adenocarcinoma. The patient was subsequently diagnosed with microsatellite instability-high cancer of unknown primary origin. Surgical removal of the affected lymph nodes achieved full remission. Chemotherapy was considered in case of recurrence or identification of the primary site. Recurrence has not occurred in three years since the surgery. However, a long-term survival without chemotherapy is rare.

Key words: cancer of unknown primary, surgery, microsatellite instability-high, long-term survival

(Intern Med 61: 3301-3308, 2022)
(DOI: 10.2169/internalmedicine.9218-21)

Introduction
Cancer of unknown primary origin (CUP) is a malignant tumor with an unknown primary site, histologically identified as a metastatic site (1). The frequency of CUP varies moderately across reports but generally ranges from 1-5% (2). Malignant tumors without a definite primary site are classified as a heterogeneous group of mixed tumors with various clinical forms.

CUP has a poor prognosis, with a median survival of 6-9 months and 2-4 months for patients with metastases confined to lymph nodes and for those with extranodal disease, respectively (3). The diagnosis and treatment of CUP remain difficult because it is a generic term for a group of unknown diseases.

We herein report a rare case of CUP in a patient who was surgically cured and survived for three years without chemotherapy.

Case Report
An 80-year-old woman was referred to her doctor with a complaint of abdominal pain. The patient did not have any relevant medical history. Interviews with the patient’s third-degree relatives also revealed no family history of cancer.

Abdominal pain was attributed to a colic attack mediated by gallstones. However, the patient was incidentally suspected of having an enlarged lymph node on the lesser curvature of the stomach based on abdominal computed tomography (CT) and was referred to our hospital for a thorough examination. Upper gastrointestinal endoscopy did not show any lesions suggestive of malignancy in the esophagus, stomach, or duodenum. Similarly, abdominal ultrasonography and endoscopic ultrasonography did not reveal any lesions suggestive of malignancy in the liver, biliary tract, or pancreas. Thus, abdominal magnetic resonance imaging (MRI) was not performed because no lesions had been detected on previous examinations.

Fluorodeoxyglucose-positron emission tomography (FDG-
PET) was performed to identify the primary organ; however, no significant accumulation was observed, except for in the lymph nodes on the lesser curvature of the stomach (#3) and the left gastric artery (#7), as had been observed on CT (Fig. 1). Blood test results did not reveal any elevated tumor markers that could identify the primary tumor (Table 1).

Thus, we performed an open lymph node biopsy to determine the histological type of the lesion. We found a hard, yellowish-white tumor. An incision of the reticulum revealed that the tumor was mildly adherent to the pancreas but could be detached. Following dissection of the surrounding tissue, we identified non-contiguous lymph nodes in the space between the tumor and gastric wall. The lymph nodes on the lesser curvature of the stomach (#3) and left gastric artery (#7) were lumped together. However, there was no evidence of adhesion to or infiltration of the surrounding organs; thus, the nodes (approximately 60 mm in size) were resected (Fig. 2a).

The results of the rapid pathological diagnosis suggested that the cancer had metastasized to the lymph nodes. Therefore, we thoroughly examined the intra-abdominal organs for neoplastic lesions; however, no abnormalities were noted. Considering the absence of disseminated nodules in the peritoneum and abnormal ascites, we performed lymphadenectomy. The patient had no postoperative complications and was discharged on day 7 post-surgery.

The tumor was diagnosed as poorly differentiated adenocarcinoma of gastrointestinal origin (Fig. 2b), and MLH1 deficiency (Fig. 2c) and microsatellite instability-high (MSI-H) cancer were determined. An immunostaining analysis revealed that the tumor was positive for markers of gastrointestinal origin, such as caudal-type homeobox2 (CDX-2) and villin (Table 2). Therefore, we performed endoscopic retrograde cholangiopancreatography to evaluate the pancreaticobiliary duct. Furthermore, the patient underwent endoscopic nasopancreatic drainage and pancreatic juice cytology. The result was benign. Colonoscopy revealed a stalked lesion in the rectum (φ15 mm), which was resected by endoscopic submucosal dissection, with a diagnosis of tubular adenoma. Repeat abdominal ultrasonography before the surgery did not show any abnormalities in the liver or gallbladder. There were no lesions in the gastrointestinal organs that were suggestive of primary cancer. Subsequently, transvaginal ultrasonography, uterine abrasion cytology, and ovarian MRI for
CUP were performed, and gynecological cancer was ruled out. Thyroid ultrasonography did not confirm any abnormalities.

FDG-PET was performed again six months and one year after the surgery to search for the primary tumor; however, no significant accumulation was found in the whole body (Fig. 3). Accordingly, the patient was diagnosed with “cancer of unknown primary cured by surgery with R0 resection.” Chemotherapy was considered to prevent recurrence, but the patient recovered completely with no recurrence or new lesions as of approximately three years postoperatively.

### Discussion

CUP is characterized by rapid progression, difficulty predicting metastatic patterns, and early dissemination (4). The pancreas, biliary tract, and lungs are the most common primary sites identified by pathological autopsy. However, the primary site cannot be identified in 20-50% of patients even after pathological autopsy (1, 5). In Greece, 884 cases of CUP were diagnosed between 1994 and 2000. The primary sites included the lungs (20%), pancreas (17%), liver or kidney (6%), colon or genitals (5%), stomach (4%), and bladder, ureter, or mammary glands (1%) (6).

FDG-PET is often used for a systemic search; however, its application for the imaging diagnosis of CUP has not yet been established. Rades et al. (7) studied 42 cases of CUP considered as a single metastatic lesion and reported a primary tumor in 43% of the cases, dissemination in 38%, and a change in treatment plan in 69% after FDG-PET.

In our patient, malignant lymphoma was initially suspected, and FDG-PET was performed for staging. However, no significant accumulation in lymph nodes was noted, except in those on the lesser curvature of the stomach and the left gastric artery. At the preoperative examination stage, malignant lymphoma was assumed to be the primary cause. Following consultation with the patient and her family, we considered chemotherapy, which warrants the identification of the histological type to determine the chemotherapy regimen.

Considering the absence of lesions on the body surface from which the tissue samples could be obtained, we performed a surgical intra-abdominal lymph node biopsy. Intraoperative findings suggested that the lesion was not adherent to the surrounding area and could be dissected. Thus, we removed the entire lesion instead of collecting a specimen. We performed a rapid pathological diagnosis of lymph node metastasis. Considering the absence of organs with a primary lesion during the subsequent thorough examination, the patient underwent only lymphadenectomy. Lymph nodes on the lesser curvature side (#3) and in the left gastric artery (#7) were present as a mass. However, neoplastic lesions were absent in the surrounding organs (stomach, pancreas, biliary tract, and liver). Thus, we considered hematogenous metastasis rather than lymphogenous metastasis.

Considering the hemodynamics, the tumor seemed to have first metastasized to #7, followed by progressive metastasis to #3, eventually forming a single mass. Based on the anatomical location of the two sites, there was presumably no inconsistency in the formation of a single mass. Despite the involvement of the liver, biliary tract, pancreas, and stomach in #7, it was difficult to identify the primary organ from the lymph nodes.

Pathological examinations, along with imaging, play an important role in identifying the primary site of CUP (8, 9). In this case, tumor cells were positive for basic markers of gastrointestinal origin, such as CDX-2 and villin, and adenocarcinoma of gastrointestinal origin was considered. Histomorphologically, there was no glandular duct formation, thus suggesting a poorly differentiated adenocarcinoma. The cells did not express MLH1 but displayed deficient mismatch repair (dMMR). dMMR adenocarcinoma of gastrointestinal origin has atypical immunohistological characteristics, thus making it difficult to determine the primary organ. In this case, CK7, claudin 18, mucin 1, and other antigens...
Figure 2. The excised specimen and its pathological features. a: Excised lymph nodes (#3 and #7) approximately 60×30 mm in size. The excised tumor is soft and elastic, surrounded by a capsule, and has been predominantly replaced by white myeloid tissue. b: High-magnification views suggest poorly differentiated adenocarcinoma (Hematoxylin and Eosin staining, ×400). c: MLH1 expression is lost (immunohistochemistry, ×400).

| Table 2. Immunohistochemistry and MSI-H Results of Excised Lymph Nodes. |
|---------------------------------------------------------------|
| **Immunohistochemistry (IHC)**                                 |
| Positive                                                      |
| CK7, CK AE1/AE3, CDX-2,                                       |
| Villin, MUC1, MUC2, claudin18, cadherin                        |
| Negative                                                     |
| EBER-ISH, estrogen receptor, GATA3, PAX8, SATB2, TTF1         |
| Equivocal                                                    |
| SMAD4 expression                                             |
| Retained                                                    |
| MLH1 expression                                             |
| Loss                                                        |
| **MSI-H**                                                   |
| Positive                                                    |
| CK7: cytokeratin7, CK AE1/AE3: cytokeratin AE1/AE3, CDX-2: caudal-type homeobox 2, MUC1: mucin 1, MUC2: mucin 2, MSI-H: microsatellite instability-high, ERER-ISH: Epstein-Barr virus encoded small RNA in situ hybridization, GATA3: GATA binding protein-3, PAX8: paired box protein-8, TTF-1: thyroid transcription factor-1, CK20: cytokeratin20, MUC5AC: mucin5AC, MLH1: MutL homolog-1 |

were positive in the pancreatic, biliary, and gastric systems, indicating that these were the primary organs. However, dMMR colorectal cancers are positive for antigens, such as CK7, which are not usually expressed in general colorectal
cancers, suggesting the possibility of a colorectal origin.

CUP cases are classified into poor and good prognosis groups (3) (Table 3). Lymph nodes, bone, and lungs are the most frequent metastatic sites, with lymph node metastasis being the most common. Lymph node metastases are more common in cervical lymph nodes (40%), whereas those in abdominal lymph nodes are relatively rare (2-6.3%) (10). Our patient was classified as having a good prognosis based on the following criteria: “single, small, resectable lesion,” “poorly differentiated cancer,” “performance status 0-1,” “normal serum lactate dehydrogenase level,” “normal serum albumin level,” “CDX-2-positive,” “no liver metastasis,” and “one metastatic organ.” However, there is no treatment plan recommended for cases confined to intra-abdominal lymph nodes.

Chemotherapy for CUP necessitates determination of the

| Good prognosis group                                                                                      | Poor prognosis group                                                                 |
|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Adenocarcinoma, female, and axillary lymph node metastasis only                                        | Liver metastasis (adenocarcinoma)                                                   |
| Serous adenocarcinoma, female, carcinomatous peritonitis, elevated CA125, and peritoneal cancer        | Peritoneal metastasis (non-papillary adenocarcinoma)                                 |
| Adenocarcinoma, male, elevated PSA, and osteogenic bone metastasis                                     | Multiple brain metastases (squamous cell carcinoma or adenocarcinoma)                |
| Squamous cell carcinoma, cervical (other than supraclavicular) lymph node metastasis                    | Multiple lung metastasis or pleural metastasis (adenocarcinoma)                      |
| Squamous cell carcinoma, inguinal lymph node metastasis                                                | Multiple brain metastases (squamous cell carcinoma or adenocarcinoma)                |
| Poorly differentiated (high-grade) neuroendocrine carcinoma                                            | Multiple osteolytic metastases (adenocarcinoma)                                     |
| Median distribution of disease (mediastinal, retroperitoneal lymph nodes, and pulmonary metastasis),  | Pelvic cavity metastasis (squamous cell carcinoma)                                   |
| male, age <50 years, and elevated beta-hCG/AFP (extra-gonadal germ cell tumor)                        |                                                                                      |
| Poorly differentiated and undifferentiated cancer                                                      |                                                                                      |
| One metastatic organ                                                                                    |                                                                                      |
| No liver metastasis                                                                                    |                                                                                      |
| Performance status 0-1                                                                                 |                                                                                      |
| Serum LDH normal                                                                                       |                                                                                      |
| Serum albumin normal                                                                                   |                                                                                      |
| Resectable lesions (single, small)                                                                     |                                                                                      |
| CK20+, CK7-, and CDX-2+                                                                                 |                                                                                      |
presence of a good or poor prognosis and the administration of standard treatment according to the primary organ cancer assumed in each group (6). However, older patients are often less tolerant to chemotherapy than younger ones because of their general condition and adverse events. In addition, they are not always treated in accordance with the guidelines (3).

MSI can be seen in two main pathologies: 1) Lynch syndrome, an inherited tumor; and 2) sporadic tumors caused by methylation of the MLH1 promoter region, a tumor suppressor gene. A total of 10-15% of sporadic colorectal and gastric cancers show MSI-H (11), and it has been reported to occur more frequently in the elderly than in younger patients (12). DNA methylation is explained by epigenetics, which is a well-established mechanism of regulating gene expression without a change in the gene sequence. Aging, chronic inflammation, and the presence of certain chemicals are thought to be triggers of epigenetics abnormalities, and aging in particular is the oldest known trigger of DNA methylation (13, 14). However, in the present case, there was no family history of cancer up to the third degree of consanguinity. As the age of the onset was later in life and because the patient had no history of cancer, it was presumed that the patient had manifested characteristics of a sporadic tumor with MSI-H due to epigenetic inactivation of MLH1 mainly caused by aging.

In 2018, pembrolizumab was approved by insurance for MSI-H solid tumors after chemotherapy resistance (15, 16), and in 2021, nivolumab was approved for insurance coverage for treating CUP (17). Therefore, in the future, if this patient relapses, we will need to consider whether pembrolizumab should be used after a cytotoxic anticancer agent or first with nivolumab.

Although five other CUP cases were reported to have survived after surgical treatment alone in Japan (18-22) (Table 4), to our knowledge, this is the first report of a successfully treated case of MSI-H CUP. Of the six cancer cases of unknown primary reported in Japan (including our case) with a long-term survival after surgery alone, four involved intra-abdominal lymph nodes. In addition, there were two

Table 4. CUP with Long-term Survival Following Surgery Alone.

| Case 1<sup>1)b</sup> | Case 2<sup>19) </sup> | Case 3<sup>2(b)</sup> | Case 4<sup>21) </sup> | Case 5<sup>22) </sup> | Case 6 (our case) |
|---------------------|----------------------|---------------------|----------------------|----------------------|----------------------|
| Age 85              | 77                   | 68                  | 76                   | 72                   | 80                   |
| Sex Male            | Male                 | Male                | Female               | Male                 | Female               |
| Past history None   | None                 | Hypertension        | None                 | None                 | None                 |
| Location            | Common hepatic trunk lymph node (#8) Subgastric pyloric lymph node (#6) Inguinal lymph node Pelvic lymph nodes Mediastinal lymph node (#2R) Lymph nodes of gastric lesser curvature (#3) | Lymph nodes of gastric lesser curvature (#3) | Common hepatic trunk lymph node (#8) | Lymph node of gastric lesser curvature (#3) | |
| Quantity 2          | 2                    | 3                   | 2                    | 2                    | 2                    |
| SUV max in FDG-PET | Not mentioned        | Not mentioned       | Not mentioned       | #3 5.7 cm            | #8 5.6 cm            |
| Elevated tumor markers | CEA (5.7 ng/mL) | None                | None                | None                 | None                 |
| Surgical formula    | Pancreatocircumduodenectomy | Lymphadenectomy | Lymphadenectomy | Lymphadenectomy | Ileocecal resection |
| Pathology           | Mucinous             | Neuroendocrine carcinoma (poorly differentiated) | Adenocarcinoma (poorly differentiated) | Tubular adenocarcinoma (moderately differentiated) | Tubular adenocarcinoma (moderately differentiated) |
| IHC (positive)      | CEA, EMA, and PAS    | CK20, CKAE1/AE3, CD56, and CAM5.2 | Not mentioned            | CK7, CK20, and CDX-2 | CDX-2, MUC1, MUC2, MUC5AC, and MUC6 |
| Tracking period (years) | 8                   | 3                   | 8                    | 5                    | 3                    |
| Recurrence Nothing  | Nothing              | Nothing             | Nothing              | Nothing              | Nothing              |

CK7: cytokeratin7, CK20: cytokeratin20, CK AE1/AE3: cytokeratin AE1/AE3, CDX-2: caudal-type homeobox-2, MUC1: mucin1, MUC2: mucin2, MUC5AC: mucin 5AC, MUC6: mucin 6, CAM5.2: cytokeratin, CEA: carcinoembryonic antigen, EMA: epithelial membrane antigen, PAS: periodic acid Schiff, CD56: cluster of differentiation 56
cases involving the common hepatic artery lymph node (#8) and gastric lesser curvature side lymph node (#3). We therefore discuss the pathogenesis of these nodes, including the left gastric artery lymph node (#7), where cancer metastasis was observed in this case.

As shown in Table 5, many cancers have lymph nodes #7 and #8 as their relay nodes, since they cover a wide area, which makes cancers likely to congregate here (23-27). In addition, even if the primary tumor is small, cannot be identified by imaging tests, or is located somewhere in the abdominal cavity, there is a high possibility that cancer cells will collect in lymph nodes #7 or #8. As #3 is a lymph node downstream of #7, we speculate that the cancer cells first metastasized from the primary tumor somewhere in the abdominal cavity to lymph node #7, metastasized to #3, and formed a mass. Although the histopathological examination of the lymph nodes removed in this case showed a “high possibility of metastasis from digestive cancer,” this is only because the lymph nodes showed characteristics similar to metastatic lesions, and the six reported cases did not involve actual metastatic lesions. In fact, the resected lesion itself may turn out to be the primary tumor on a future analysis. To further elucidate the cytological characteristics of CUP, it is necessary to continue to accumulate cases and investigate the clinical characteristics.

Regarding post-treatment surveillance, there are no data showing that identifying the primary lesion improves the prognosis, and it is therefore recommended that patients diagnosed with CUP not undergo extensive examinations to identify the primary lesion after the start of treatment (3). However, this recommendation is only for patients with residual disease and not applicable to those with curative diseases; the surveillance algorithm for CUP in a curative state has not been established; thus, future studies should focus on determining what modality to use and how to conduct surveillance.

Notably, there are CUP cases that can be cured by surgery, and in these cases, the use of immune checkpoint inhibitors (ICIs), combined with chemotherapy, should be considered, if indicated by the results of next-generation sequencing and the social background and circumstances of the patient. This case highlights the importance of this approach. To date, there have been only a few reports of patients with CUPS who have survived for a considerable period, and there are also a few reports examining the frequency of dMMR/MSI-H CUP cases. Thus, it is important to accumulate additional, similar cases in the future.

**Conclusion**

We encountered a case of dMMR/MSI-H-positive CUP in an elderly woman with a prolonged survival following surgical treatment alone. Surgery should be actively considered in cases with a possible cure for CUP. Furthermore, the use of ICIs, which allow for cross-organ treatment by searching for dMMR/MSI-H, is an option.

The authors state that they have no Conflict of Interest (COI).

**References**

1. Pavlidis N, Pentheroudakis G. Cancer of unknown primary site. Lancet 379: 1428-1435, 2012.
2. Muir C. Cancer of unknown primary site. Cancer 75: 353-356, 1995.
3. Ettenger DS, Varadhachary GR, Bowles DW, et al. NCCN clinical practice guidelines in oncology. Occult primary (cancer of unknown primary [CUP]) version 2 J Natl Compr Canc Netw 12: 969-974, 2019.
4. Pavlidis N. Cancer of unknown primary: biological and clinical characteristics. Ann Oncol 14: iii11-iii18, 2003.
5. Blaszyk H, Hartmann A, Bjornsson J. Cancer of unknown primary: clinicopathologic correlations. APMIS 111: 1089-1094, 2003.
6. Pentheroudakis G, Briasoulis E, Pavlidis N. Cancer of unknown primary site: missing primary or missing biology? Oncologist 12: 418-425, 2007.
7. Rades D, Kuhnel G, Wildfang L, Börner AR, Schmoll HJ, Knapp W. Localized disease in cancer of unknown primary (CUP): the value of positron emission tomography (PET) for individual therapeutic management. Ann Oncol 12: 1606-1609, 2001.
8. Bitran JD, Ultmann JE. Malignancies of undetermined primary origin. Dis Mon 38: 213-260, 1992.
9. Hewitt MJ, Anderson K, Hall GD, et al. Women with peritoneal...
carcinomatosis of unknown origin: efficacy of image-guided biopsy to determine site-specific diagnosis. BIOG 114: 46-50, 2007.

10. Didolkar MS, Fanous N, Elias EG, Moore RH. Metastatic carcinomas from occult primary tumors. A study of 254 patients. Ann Surg 186: 625-630, 1977.

11. Aaltoenen LA, Peltomäki P, Leach FS, et al. Clues to the pathogenesis of familial colorectal cancer. Science 260: 812-816, 1993.

12. Miyakura Y, Sugano K, Konishi F, et al. Methylation profile of the MLH1 promoter region and their relationship to colorectal carcinogenesis. Genes Chromosomes Cancer 36: 17-25, 2003.

13. Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 7: 536-540, 1994.

14. Ushijima T, Okochi-Takada E. Aberrant methylations in cancer cells: where do they come from? Cancer Sci 96: 206-211, 2005.

15. Marabelle A, Le DT, Ascierto PA, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase IIKEYNOTE-158 study. J Clin Oncol 38: 1-10, 2020.

16. Le DT, Kim TW, Van Cutsem E, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. J Clin Oncol 38: 11-19, 2020.

17. Tanizaki J, Yonemori K, Akiyoshi K, et al. Open-label phase II study of the efficacy of nivolumab for cancer of unknown primary. Ann Oncol 33: 216-226, 2022.

18. Kimura A, Ueta T, Suzuki K, Minagi S, Takeuchi T. A case of intra-peritoneal lymph node metastasis of unknown primary cancer. J Jpn Surg Assoc 66: 1757-1761, 2005 (in Japanese).

19. Hisamori S, Okabe H, Yoshizawa A, Sakai Y. A case of long-term recurrence-free poorly differentiated neuroendocrine carcinoma of lymph nodes treated by surgical resection without any chemotherapy. Int J Clin Oncol 15: 493-496, 2010.

20. Yamada M, Gotoh S, Minagawa T. Experience of long term survival in a case with surgery for cancer metastasis to brain and mediastinal lymph node from unknown primary site. Kyobu Geka (Jpn J Thorac Surg) 64: 570-573, 2011 (in Japanese).

21. Eisaku I, Shigeki W, Yuki F, Masahiro I, Katsuhiko Y. A case of long-term survival without recurrence from an unknown primary carcinoma after resection of lymph node metastasis. J Gastroenterol 111: 1990-1996, 2004 (in Japanese).

22. Matsuzawa H, Aoki J, Munezo S, et al. Unknown primary cancer with ileocecal lymph node metastasis - a case report. J Jpn Surg Assoc 80: 1162-1167, 2019 (in Japanese).

23. Japan Esophageal Society. Japanese classification of esophageal cancer, 11th ed. Esophagus 14: 1-65, 2017.

24. Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma. 15th ed. Kanehara, Tokyo, 2017 (in Japanese).

25. Japanese Society of Hepato-Biliary-Pancreatic Surgery. General Rules for Clinical and Pathological Studies on Cancer of the Biliary Tract. 7th ed. Kanehara, Tokyo, 2021 (in Japanese).

26. Japan Pancreas Society. General Rules for the Study of Pancreatic Cancer. 7th ed. Kanehara, Tokyo, 2020 (in Japanese).

27. Liver Cancer Study Group of Japan, General Rules for Clinical and Pathological Studies on Cancer of Primary Liver Cancer. 6th ed. Kanehara, Tokyo, 2019 (in Japanese).

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (https://creativecommons.org/licenses/by-nc-nd/4.0/).