Cancer of unknown primary histologically, genetically and spatially diagnosed as left ovary-derived cancer: A case report

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Abstract. Cancer of unknown primary (CUP) is a heterogeneous syndrome of metastatic cancer in which the primary site cannot be determined even after a standard and comprehensive search. The present report describes a case in which the spatial distribution of the lymph node metastases contributed to the identification of the primary site. While the standard workup did not identify the primary tumor, genomic profiling analysis was useful in therapeutic management. A 68-year-old woman presented with a cancerous pleural effusion (adenocarcinoma). The primary site could not be identified, and the pleural effusion resolved spontaneously. After 11 months, the patient had elevated Krebs von den Lungen-6 and cancer antigen 125 levels, and multiple enlarged lymph nodes. Pathological diagnosis based on a biopsy sample of the para-aortic lymph nodes indicated that the tumor was a high-grade serous carcinoma of possible gynecological organ origin. The patient underwent surgery, including hysterectomy, bisalpingo-oophorectomy and lymph node dissection. Although there were no primary sites in the gynecological organs, marked lymphovascular invasion was found around the left ovary, suggesting a left ovary-derived tumor. Genetic testing revealed a high loss of heterozygosity score and high tumor mutational burden (TMB). The patient received paclitaxel and carboplatin therapy followed by a poly ADP-ribose polymerase inhibitor as regimens for ovarian cancer and achieved complete remission. The unique course of the disappearance of the effusion and the absence of tumor in the adnexa might be associated with the high immunogenicity of the tumor characterized by the high TMB. This case may provide insights into the pathogenesis of CUP.

Introduction

Cancer of unknown primary (CUP) is a heterogeneous syndrome of metastatic cancers in which the primary site cannot be determined after a standard and comprehensive search, and accounts for 1-5% of all newly diagnosed malignancies (1,2).

CUP falls into either the clinicopathologically 'favorable' (~15% of cases) or 'unfavorable' (~85% of cases) subgroups (3-5). The former includes adenocarcinoma of the axillary lymph nodes, papillary serous carcinoma of the peritoneum (redefined as peritoneal cancer), squamous cell carcinoma of the cervical lymph nodes and extragonadal germ cell tumors in young men, which are derived from the middle of the body (4). For these patients, specific therapeutic management can be provided, and satisfactory local control and prolonged survival can be expected, with a median survival time of >24 months (6). On the other hand, the unfavorable subset (majority of patients) includes patients with adenocarcinoma metastasis to the liver or other organs, poorly differentiated carcinoma and squamous cell carcinoma of the abdominal cavity (1). These patients are usually treated with empiric chemotherapies based on platinum and taxanes (7) but are generally not chemosensitive and have a poor prognosis, with a median survival time of 5-8 months (4,8).

Delayed initiation of treatment and a tendency to choose empiric chemotherapy are associated with a poor prognosis (4). Therefore, a thorough workup at the time of diagnosis is important in CUP. The distribution and histology of the cancer are important for estimating the primary site. In addition to image-based and immunohistochemical examinations, the cancer genomic profiling (CGP) test has been used to estimate primary sites and identify potential targets for personalized

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Abbreviations: CUP, cancer of unknown primary; CGP, cancer genomic profiling; PET-CT, positron emission tomography-computed tomography; HGSC, high-grade serous carcinoma; TMB, tumor mutational burden; TMB-H, TMB-high; ICI, immune checkpoint inhibitor; HRP, homologous recombination-proficient; HRD, homologous recombination-deficiency; TC therapy, paclitaxel-carboplatin combination therapy; SUV, standardized uptake value; CA125, cancer antigen 125; PARP, poly ADP-ribose polymerase

Key words: CUP, HGSC, gynecological origin, genetic testing, ovarian cancer

therapies (9,10). Several studies using next-generation sequencing have demonstrated that molecular profiling for CUP is useful for predicting the tissue of origin (9,11-14). However, to the best of our knowledge, it has not been determined whether genomic profiling in CUP can help in providing tissue-specific therapies, including targeted therapies, to improve survival. A prospective randomized phase II trial (CUPISCO trial; NCT03498521) is currently underway to investigate the efficacy of CGP in CUPs; however, there are issues, such as insufficient sample volume and cases of misdiagnosis (15,16).

In the present study, a case of CUP is reported in which genomic profiling and lesion distribution were used to infer the primary organs of cancer and to determine a suitable therapeutic strategy.

Case report

A 68-year-old woman, gravida 1 para 1, who had a history of allergic dermatitis for 2 years and had been taking prednisone irregularly, presented to their primary care physician (Yokohama, Japan) in August 2020 with dyspnea on exertion. Right pleural effusion was observed, and 1,600 ml pleural fluid was aspirated by puncture. Papanicolaou staining was performed on the pleural fluid as previously described (17), and the cytological examination revealed Class V adenocarcinoma (Fig. 1A) according to the Papanicolaou classification (18). Upper and lower gastrointestinal endoscopy and gynecological examination were performed but the primary tumor was not identified. In October 2020, the patient was referred to the Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo (Tokyo, Japan). By then, the pleural effusion had resolved spontaneously. Positron emission tomography-computed tomography (PET-CT) revealed mild fluorodeoxyglucose accumulation in the left predominant para-aortic and left internal iliac lymph nodes; however, this was considered to be a reactive change. At 11 months after the initial visit, the Krebs von den Lungen-6 level measured using chemiluminescent enzyme immunoassay (CLEIA) was elevated (1,243 U/ml; normal range, 0-464 U/ml), and the patient was referred back to the hospital for a close examination of the primary site. PET-CT revealed abnormal accumulation in the right axillary lymph node with a maximum standardized uptake value (SUV) of 3.8, and further accumulation in the peripancreatic, para-aortic, mesenteric, sacral and bilateral external iliac lymph nodes with a maximum SUV of 7.2. All lymph node accumulations were considered to indicate metastatic lymph nodes (Fig. 1B).

Due to elevated cancer antigen 125 (CA125) levels (309 U/ml; normal range, 0-35 U/ml) measured using CLEIA and the distribution of the metastatic lymph nodes, gynecological cancer was suspected; however, pelvic magnetic resonance imaging showed no obvious primary tumor and multiple uterine fibroids (Fig. 1C). The cervical and endometrial cytologies were negative. Fine-needle aspiration cytology of the right axillary lymph node revealed metastatic adenocarcinoma (data not shown). For histological diagnosis, a laparoscopic para-aortic lymph node biopsy was performed. Hematoxylin-and-eosin (H&E)-stained slides were made from formalin-fixed paraffin-embedded (FFPE) blocks as previously described (17). Immunohistochemical staining was performed using the Ventana BenchMark XT automated staining system (Roche Diagnostics) according to the manufacturer's protocol, as previously described (19). The primary antibodies used were as follows: Cytokeratin 7 (cat. no. 790-4462; clone SP52; Roche Diagnostics), estrogen receptor (EP1, Envision FLEX-ER, Dako; Agilent Technologies, Inc.), paired box 8 (clone 10336-1-AP; Proteintech Group, Inc.), p53 (clone DO-7; Roche Diagnostics), Wilm's tumor 1 (clone 6F-H2; Dako; Agilent Technologies, Inc.), GATA binding protein 3 (clone L50-823; Biocare Medical, LLC), cytokeratin 20 (clone SP33; Roche Diagnostics) and D2-40 (clone D2-40; Dako; Agilent Technologies, Inc.). All the cytopathological, histological and immunohistochemical images were examined and captured using a light microscope (BX51; Olympus Corporation). The tumor was diffusely positive for cytokeratin 7, estrogen receptor, paired box 8, p53 and Wilms tumor 1, whereas it was negative for GATA binding protein 3 and cytokeratin 20 (Fig. 2A). The histological and immunohistochemical diagnosis was of a high-grade serous carcinoma (HGSC) possibly derived from the gynecological organs, based on the WHO Classification (20).

Based on the distribution of the tumors, high CA125 levels, histology and immunostaining results, HGSC of the ovary or fallopian tubes was suspected to be the primary carcinoma. For the purpose of diagnosis and tumor debulking, a total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy, and partial omentectomy were performed (Fig. 2B). Pathological examination of the surgical specimen, including histological assessment of H&E-stained slides of FFPE tissues, revealed that there were no primary tumors in the parenchyma of the ovaries or fallopian tubes, and also no peritoneal dissemination, with negative peritoneal washing cytology, or serous tubal intraepithelial carcinoma; however, there were multiple metastatic adenocarcinomas in para-aortic and pelvic lymph nodes. Although some lymph node metastases were present on both sides, including the para-aortic, sacral and external iliac lymph node metastases, some were only on the left, including the common iliac, internal iliac and obturator lymph node metastases. Combining preoperative imaging findings and pathological diagnosis, the distribution of multiple lymph node metastases was recorded as predominantly left-sided (Fig. 2C). Although no apparent primary site was identified, lymphatic invasion of HGSC was microscopically detected (Fig. 2D) within the left ovarian parenchyma, and given the distribution of the lymph node metastases, it was suggested that the tumor was left ovary-derived (Fig. 2D). Reevaluation of the pleural fluid cytology image at this point revealed a high nucleus-to-cytoplasmic ratio, which was consistent with an HGSC-like appearance (21). As no primary tumor had been identified, the tumor was diagnosed as a CUP. A CGP test was conducted to diagnose CUP. FoundationOne® CDx (Foundation Medicine, Inc.) is a qualitative next-generation sequencing-based *in vitro* diagnostic test performed by Foundation Medicine, Inc. (22) that showed 14 somatic variants (Table I), including three likely pathogenic variants [*TP53* (p.G266R), *CIC* (p.E1263Gfs*78) and *PBRM1* (p.I223Yfs*36)], and nine gene amplifications of *CCND2, CSF3R, FGF23, FGF6, KDM5A, MYC, PIK3C2G, RAD52* and *RICTOR* (Table II). The tumor was microsatellite-stable with a tumor mutational burden

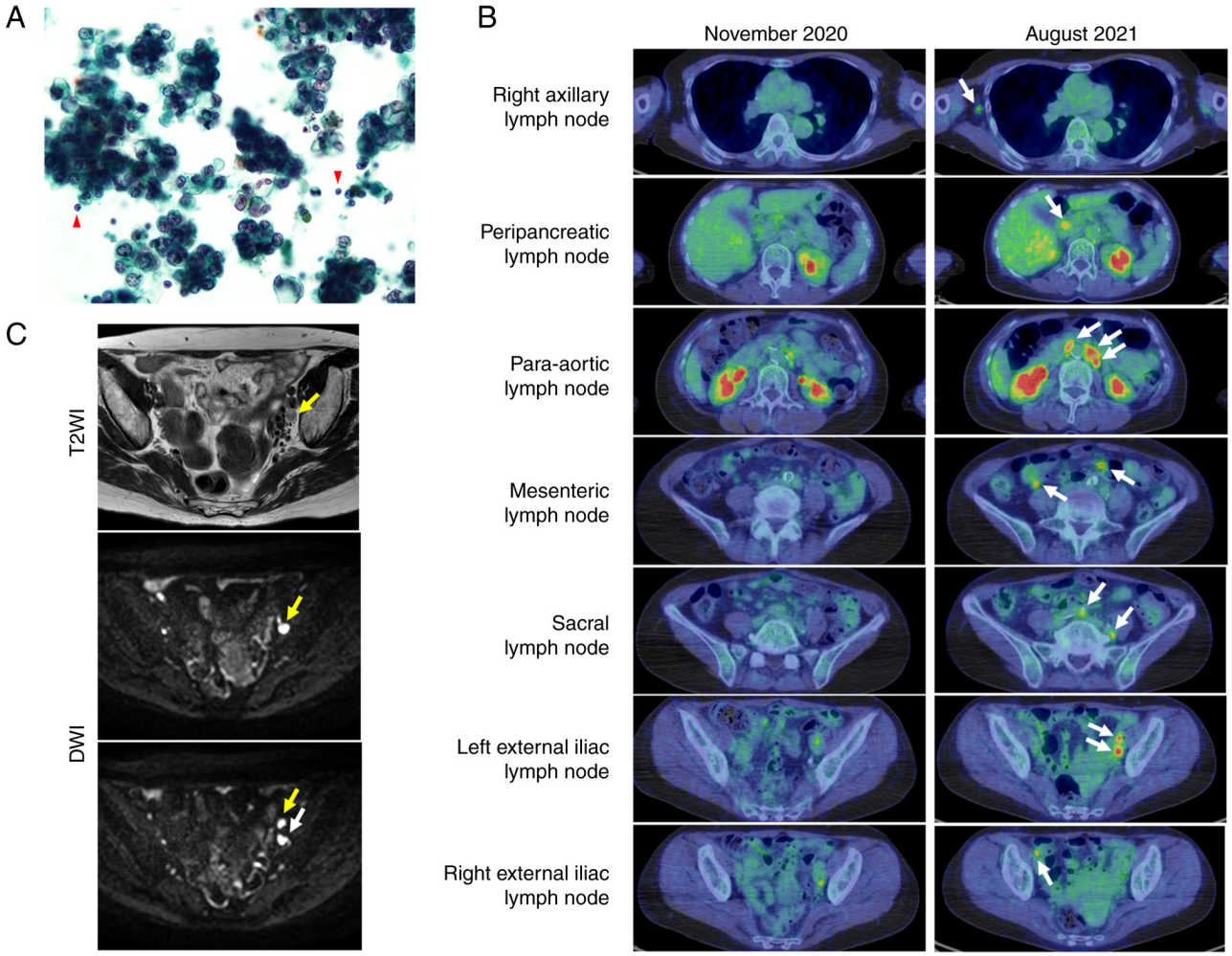

Figure 1. Pleural fluid cytology and imaging diagnosis. (A) Pleural fluid cytology (Papanicolaou stain; magnification, x400). A large number of atypical cells with enlarged nuclei, thickened nuclear periphery and conspicuous nucleoli were observed in pseudopapillary or spherical aggregates or sporadically when compared with mesothelial cells, neutrophils or lymphocytes. Cyst-like spaces were present, although mucin was not evident. Lymphocytes indicated by the red arrows were used as controls for comparison. (B) ^{18}F-FDG PET-CT in November 2020 (left) and August 2021 (right). PET-CT images captured at the primary visit indicated no conspicuous ^{18}F-FDG accumulation in the lymph nodes; however, 9 months later, lymph node enlargement with ^{18}F-FDG accumulation was observed at the sites indicated by the white arrows, and multiple lymph node metastasis was suspected. (C) Contrast-enhanced magnetic resonance imaging. Arrows indicate enlarged left obturator (white) and external iliac (yellow) lymph nodes. The upper DWI image is the same slice as the T2WI image. DWI, diffusion-weighted imaging; ^{18}F-FDG, ^{18}F-fluorodeoxyglucose; PET-CT, positron emission tomography-computed tomography; T2WI, T2-weighted imaging.

(TMB) of 10.1/Mb (high) and a loss of heterozygosity (LOH) score of 23%. Immune checkpoint inhibitors (ICIs) were recommended based on the tumor being TMB-high (TMB-H) and poly ADP-ribose polymerase (PARP) inhibitors were recommended based on the high LOH score. The tumor distribution, pathological and immunohistochemical examinations, and genomic examinations suggested gynecological organ-derived HGSC (left ovary-derived being suspected). Myriad MyChoice CDx® (Myriad Genetics, Inc.; protocol details not available), a next-generation sequencing-based *in vitro* diagnostic test performed by Myriad Genetics, Inc., revealed that the tumor was homologous recombination-proficient (HRP) with a homologous recombination-deficiency (HRD) score of 41 and no tumor *BRCA1/2* mutation.

The tumor had a high TMB, and ICIs should therefore have been considered; however, the HRD score was 41 (relatively high among HRP tumors) and the LOH score was also high, close to that of the HRD tumor (HRD score ≥42). In addition, HGSC generally has a high response rate to platinum agents and PARP inhibitors are effective only while tumors are platinum-sensitive (23-25). Therefore, it was decided to start a combination therapy of paclitaxel and carboplatin (TC therapy; paclitaxel 175 mg/m^2 and carboplatin AUC 6 mg/ml/min every 3-4 weeks) and use niraparib (200 mg/body) as maintenance therapy. After eight courses of TC therapy, the CA125 level was markedly reduced from 247 U/ml to 16 U/ml (Fig. 3), and the patient achieved complete remission. Based on the HRD score of 41, the patient was switched to niraparib maintenance therapy according to the treatment protocol for ovarian cancer (25). The patient has been followed up every month since August 2022, when niraparib was started, and will continue to be followed up at the same intervals. The patient has been relapse-free as of July 2023.

Discussion

In the present case, the diagnosis was delayed by the spontaneous resolution of the cancerous pleural effusion. However,

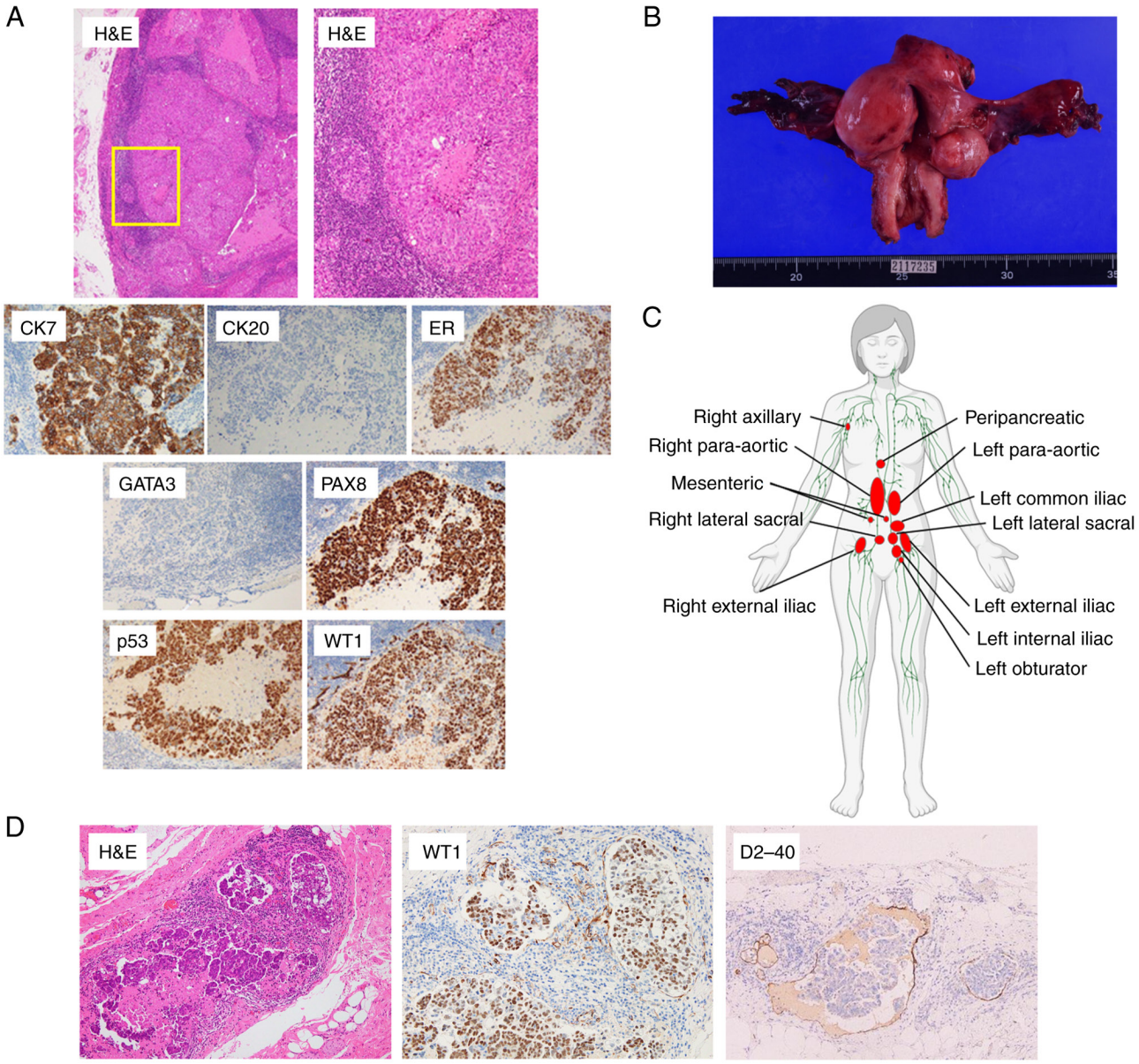

Figure 2. Distribution and pathological diagnosis of cancer of unknown primary. (A) Pathological examination of para-aortic lymph node biopsy. The upper left image shows H&E staining (magnification, x40). The upper right image is a magnified image of the area enclosed by the yellow square in the upper left image (magnification, x100). Sheets of cancer cells with necrotic foci were observed. The cancer cells had enlarged and irregularly shaped nuclei with distinct nucleoli. The lower images show immunohistochemical analysis of CK7, CK20, ER, PAX8, p53 and WT1 (all magnification, x200). (B) Macroscopic image of surgically removed uterus and bilateral adnexa. The unit on the scale is 1 mm. (C) Distribution of metastatic lymph nodes determined by pathological diagnosis of surgically removed lymph nodes and CT, MRI and PET-CT images. Metastatic lymph nodes are shown in red. This figure was created by the author using BioRender with permission to reproduce images from BioRender.com. Reprinted from 'Circulatory system (female, lymphatic)', by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates. (D) Pathological examination of the left ovary. Adenocarcinoma was present in lymphatic vessels. The morphology of tumor clusters was similar to the clusters observed in the pleural fluid cytology specimens. H&E staining (left) and D2-40 (right) magnification, x100; WT1 (center) magnification, x200. CK7, cytokeratin 7; CK20, cytokeratin 20; D2-40, podoplanin; ER, estrogen receptor; PAX8, paired box 8; WT1, Wilms tumor 1; PET-CT, positron emission tomography-computed tomography.

CUP was classified as cancer of ovarian origin based on the marked lymphatic invasion around the left ovary, the distribution of the lymph node metastases, the genomic information and the immunohistochemical data, and the patient was able to receive specific treatment for primary ovarian cancer.

The biological nature of CUP remains largely unknown. The most popular hypothesis is that it is a metastatic tumor arising from an undetectable primary tumor due to regression, dormancy and small size (26,27). Another hypothesis is that it is a single metastatic tumor without a primary tumor (3). The present case is unique in that there was no primary lesion in the uterus and adnexa, accompanied by only lymphatic invasion within the left ovary. These findings suggest that the primary tumor in the left ovary or fallopian tube might either be small or regressed spontaneously.

An important observation in this case was the disappearance of the malignant pleural effusion. To the best of our knowledge, the phenomenon of spontaneous resolution of malignant pleural effusions has not yet been reported, and its precise mechanism remains unresolved. A similar

Table I. Somatic variants.

Gene	Site	cDNA variation	Amino acid substitution	Clinical significance
TP53	17p13.1	c.796G>A	p.G266R	Likely pathogenic
CIC	19q13.2	c.3786_3793del	p.E1263Gfs*78	Likely pathogenic
EP300	22q13.2	c.2831C>T	p.A944V	-
ERBB2	17q12	c.3149C>T	p.S1050L	-
FOXL2	3q22.3	c.914C>A	p.P305Q	-
GNAS	20q13.32	c.*42+13068G>C	-	-
JAK1	1p31.3	c.1059_1082del	p.D353_R360del	-
MAF	16q23.2	c.543_544insTAC	p.Y181_H182insY	-
MPA3K13	3q27.2	c.1567A>G	p.I523V	-
MED12	Xq13.1	c.2704G>T	p.V902L	-
NFKBIA	14q13.2	c.797_805del	p.Q266_Q268del	-
NOTCH1	9q34.3	c.5422G>A	p.D1808N	-
NTRK2	9q21.33	c.1752G>C	p.L584F	-
PBRM1	3p21.1	c.666_679del	p.I223Yfs*36	Likely pathogenic

Summary of gene mutations concluded from next-generation sequencing analysis (FoundationOne® CDx). The tumor mutational burden was 10.1/Mb and the tumor was microsatellite stable. The mean read depth was 1,024 reads. ins, insertion; del, deletion.

Table II. Copy number alterations.

Gene	Site	Copy number alteration	Copy number	Clinical significance
CCND2	12p13.32	Amplification	10	-
CSF3R	1p34.3	Amplification	7	-
FGF23	12p13.32	Amplification	10	-
FGF6	12p13.32	Amplification	10	-
KDM5A	12p13.33	Amplification	10	Likely pathogenic
MYC	8q24.21	Amplification	7	Pathogenic
PIK3C2G	12p12.3	Amplification	7	-
RAD52	12p13.33	Amplification	10	-
RICTOR	5p13.1	Amplification	10	Pathogenic

Summary of copy number alterations concluded from next-generation sequencing analysis (FoundationOne® CDx). The mean read depth was 1,024 reads.

phenomenon, in which a pleural effusion appears with ovarian malignancy and disappears with the disappearance of the ovarian tumor, is known as pseudo-Meigs' syndrome (28,29). In the present case, if the malignant pleural effusion had disappeared with the spontaneous resolution of the primary ovarian tumor, it would be consistent with the pathogenesis of pseudo-Meigs' syndrome.

The reason for the disappearance of the primary tumor was subsequently considered. The patient was being administered systemic exogenous corticosteroids around the time the pleural effusion appeared. It is possible that the systemic administration of steroids suppressed cancer immunity (30). Furthermore, the tumor was TMB-H. HGSC is characterized by copy number alterations with low TMB (31-33). TMB-H tumors are characterized by high levels of neoantigens and immunogenicity (34). One hypothesis is that the patient developed highly immunogenic ovarian cancer, but that cancer immunity was suppressed during steroid administration, resulting in pseudo-Meigs' syndrome associated with ovarian cancer. Subsequent reactivation of immunity by steroid withdrawal may have triggered shrinkage of the primary tumor and disappearance of the pleural effusion.

In the present case, genomic data suggested that PARP inhibitors could be expected to be effective as maintenance therapy after TC therapy. In addition, the patient had a TMB-H tumor and was expected to benefit from ICIs. Although the patient was treated with TC therapy followed by maintenance therapy with a PARP inhibitor based on the treatment regimen for primary ovarian cancer, the efficacy of immunotherapy in CUP has been demonstrated in a phase II trial (35) and is expected to become more widespread in the future. Approximately one-third of patients with CUP have a tumor

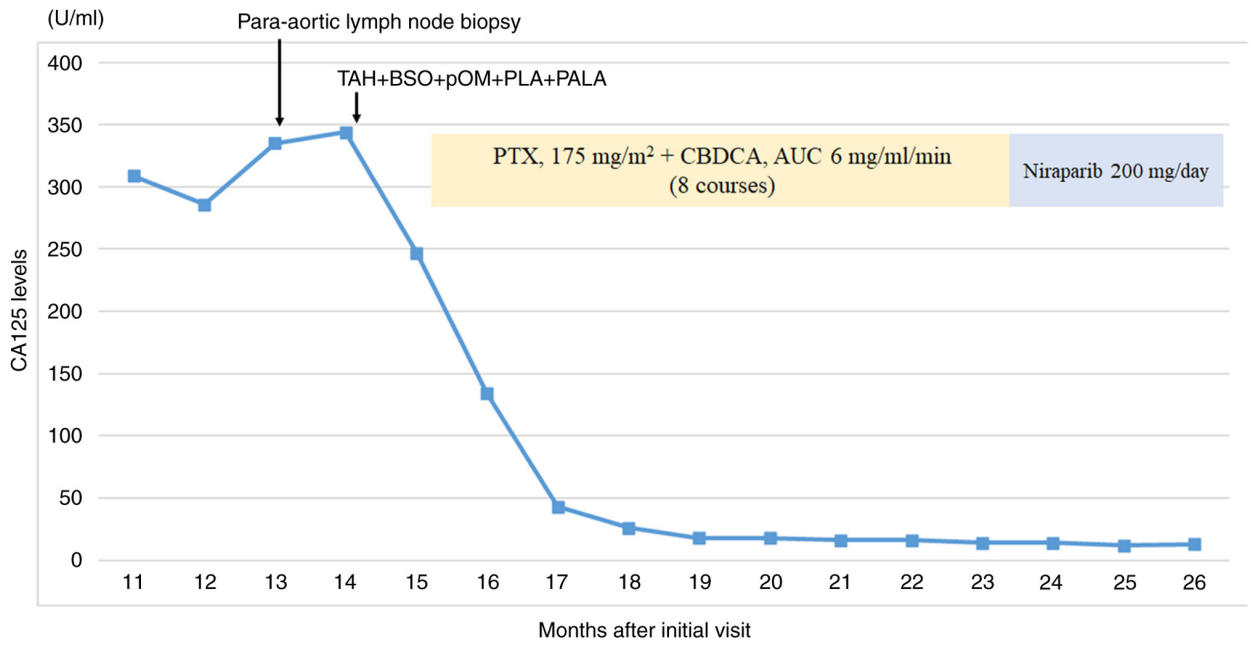

Figure 3. Summary of clinical course and CA125 levels. No recurrence was observed during the course of the treatment by contrast-enhanced computed tomography, and paclitaxel and carboplatin therapy markedly reduced CA125 levels. After 8 courses of paclitaxel-carboplatin combination therapy, the patient achieved complete remission. Since then, the patient has received niraparib maintenance therapy and has been relapse-free. TAH, total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; pOM, partial omentectomy; PLA, pelvic lymphadenectomy; PALA, paraaortic lymphadenectomy; PTX, paclitaxel; CBDCA, carboplatin; AUC, area under the curve; CA125, cancer antigen 125.

proportion score >1% for programmed cell death 1-ligand 1, and antitumor immunity-related gene expression in CUP have been reported to be comparable to those in ICI-responsive malignancies (36,37). The pleural effusion appeared at the time of steroid administration and the tumor was a TMB-H tumor, which is rare for ovarian HGSC (31,32). This may suggest that the pathophysiology of CUP in the present case report is highly immunogenic.

The present study had some limitations. First, the hypothesis of pseudo-Meigs' syndrome associated with ovarian cancer was proposed as a pathogenesis of CUP; however, it is not possible to prove this hypothesis since there was no evidence of a tumor in the left ovary. Second, based on the high LOH score, a PARP inhibitor was used after TC therapy; however, it was not possible to evaluate the response to the PARP inhibitor, as the TC therapy resulted in complete remission. Long-term observations are needed in the future to investigate the effects of PARP inhibitors and ICIs.

In the present case, lymphatic invasion, the distribution of the lymph node metastases, genomic analysis and immunohistological analysis suggested CUP of left ovarian origin, and specific therapy for ovarian cancer was provided. This unique course, which was characterized by the appearance and disappearance of the CUP, may have been associated with the immune response.

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Availability of data and materials

The sequencing datasets generated and/or analyzed during the current study are not publicly available as the Myriad MyChoice CDx® and FoundationOne® CDx reports are all the raw data that Foundation Medicine, Inc., and Myriad Genetics, Inc. can provide. All content from the reports has been provided in the manuscript. It has been confirmed with Chugai Pharmaceutical Co., and Myriad Genetics, Inc., that no other data will be provided. The other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HH and AT conceived and designed the study. HH, AT, HR, AM, AN, TA, SE, YM, KS, MUM and YO obtained data and treated this patient. HH analyzed the data and drafted the manuscript. HR analyzed the data using pathological methods. HH and HR confirm the authenticity of the pathological data. HH and AT confirm the authenticity of all other raw data. AT, HR, AM, TA, AN, SE, YM, KS, MUM and YO revised the manuscript before submission. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

This case received standard clinical treatment as a cancer of unknown primary equivalent to ovarian cancer. For this report, in accordance with the Act on the Protection of Personal Information in Japan (38), this study was approved by the Institutional Ethics Committee of The University of Tokyo (approval no. G0683; Tokyo, Japan).

Patient consent for publication

The patient provided written informed consent for publication of any associated data and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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