Primary ovary choriocarcinoma: individual DNA polymorphic analysis as a strategy to confirm diagnosis and treatment

Pedro Exman,[1] Tiago Kenji Takahashi,[1] Gilka F. Gattás,[2] Vanessa Dionisio Cantagalli,[2] Cristina Anton,[3] Fernando Nalesso,[4] Maria Del Pilar Estevez Diz[1]

[1]Department of Radiology and Oncology, Instituto do Câncer do Estado de São Paulo, Faculdade de Medicina da Universidade de São Paulo; [2]Department of Legal Medicine, Faculdade de Medicina da Universidade de São Paulo; [3]Department of Gynecology and Obstetrics, Instituto do Câncer do Estado de São Paulo, Faculdade de Medicina da Universidade de São Paulo; [4]Department of Pathology, Instituto do Câncer do Estado de São Paulo, Faculdade de Medicina da Universidade de São Paulo, Brazil

Abstract

Primary choriocarcinoma of the ovary is rare. Furthermore, this tumor can arise from gestational tissue or pure germ cells of the ovary, with the latter resulting in non-gestational choriocarcinoma. While the clinical characteristics and histology of both tumor types are identical, differentiation of these tumors is necessary for effective treatment. One strategy for the differentiation of these tumors types is to assay for the presence of paternal DNA. Accordingly, in the present case, a patient with primary choriocarcinoma of the ovary with a non-gestational origin was confirmed by DNA analysis. The patient subsequently exhibited an excellent response to chemotherapy, and following surgery, achieved complete remission. A pathological analysis of surgical specimens further confirmed the absence of tumor.

Introduction

Primary choriocarcinoma of the ovary can arise from gestational tissue or pure germ cells of the ovary. In the latter case, it is referred to as non-gestational choriocarcinoma. The estimated incidence of gestational choriocarcinomas of the ovary is 1:369,000,000 pregnancies, while non gestational choriocarcinomas correspond to less than 0.6% of ovarian germ cell tumors,[1,2] making this neoplasm very rare. Moreover, both gestational and non-gestational diseases exhibit identical clinical manifestations and histology. However, the presence of paternal DNA in the gestational choriocarcioma can differentiate the two tumor types. Correspondingly, these tumor types should be considered distinct entities with distinct therapeutic approaches, chemotherapy regimens, and prognosis associated with each disease.

Objective

To present a case report of a patient with primary choriocarcinoma of the ovary with non-gestational origin that was confirmed by DNA analysis and exhibited an excellent response to chemotherapy.

Case Report

A 24-year-old woman presented with progressive headaches, global weakness, and shortness of breath over the previous three months. A complete blood cell analysis detected a hemoglobin value of 6.0 g/dL associated with microcytosis and hypochromia. As a result, the patient was initially treated with oral ferrous sulfate. However, the patient subsequently experienced a worsening of symptoms, and after one month, presented with pain in the hypogastrium, constipation, and menstrual changes. Her medical history included an abortion followed by curettage at age 19, as well as a history of smoking.

A transvaginal ultrasound previously showed no significant changes in the uterus or ovary. However, a complex heterogeneous structure was localized to the Douglas cavity with mixed content and measured 11.5×9.3×7.4 cm.

The patient underwent computed tomography (CT) of the chest, abdomen, and pelvis. A complex heterogeneous mass in annexial topography, predominantly to the left, was found to measure 12.0×8.0×8.1 cm. This mass was responsible for forward displacement of the uterus without defined limits to it. In addition, the mass was in close proximity to the colon wall, bladder, and small bowel. A chest CT detected multiple pulmonary nodules, the largest measuring 3.7×3.6 cm, and mediastinal and peri-esophageal lymphadenopathy up to 3.5×3.5 cm (Figure 1). An enlarged liver was also observed, with a perfusional disturbance associated with a non-specific, round nodule measuring 2.8 cm along its greatest diameter present in segment IV B. The radiologist of our service suspected that the mass originated in the uterus, thereby suggesting the presence of a gestational trophoblastic neoplasm.

One week after the CT scan, the patient was referred to the emergency room with nausea, vomiting, and an absence of bowel movements constituting bowel obstruction. Blood tests detected a hemoglobin (Hgb) value of 5.0 g/dL, a beta human chorionic gonadotropin (β-hCG) value of 675,713 mIU per milliliter (normal value less than 5), a lactate dehydrogenase value of 628 U/L, an alpha-fetoprotein value of 1.0 U/mL, a cancer antigen 125 (CA-125) value of 124.4 U/mL, and a carcinoembryonic antigen (CEA) value of 3.7 U/mL.

A cervical pap smear exhibited a benign cytology, and a pelvic mass biopsy was diagnosed as pure choriocarcinoma without other cellular elements. An immunohistochemical panel was performed and the samples analyzed were positive for β-hCG, 35BH11, human placental alkaline phosphatase oncofetal antigen (PLAP) focal and placental lactogen rare cells. In contrast, samples were negative for alpha fetoprotein, CD30 antigen, WT-1 antigen, and TTF-1 antigen.

Biopsy material was also subjected to individual DNA polymorphic analysis to verify the

Correspondence: Pedro Exman, Department of Radiology and Oncology, Instituto do Câncer do Estado de São Paulo, Faculdade de Medicina da Universidade de São Paulo, Rua Jesuíno Arruda 325 apto 92, 04532-080 Sao Paulo, Brazil.
Tel. +55.119.91764565 - Fax: +55.113.8932686
E-mail: pedroexman@hotmail.com

Contributions: PE, conception, acquisition of data, analysis and interpretation of data, revising and final approval of the article; TKT, analysis and interpretation of data, revising and final approval of the article; CA, acquisition of data, drafting, revising and final approval of the article; FNA, acquisition of data, revising and final approval of the article; GFG, acquisition of data, drafting, analysis and interpretation of data, revising and final approval of the article; VC, acquisition of data, revising and final approval of the article; MDPED, conception, acquisition of data, analysis and interpretation of data, drafting, revising and final approval of the article

Conflict of interests: the authors declare no potential conflict of interests.

Key words: ovarian choriocarcinoma, gestational choriocarcinoma, pattern analysis.

Received for publication: 17 January 2013.
Revision received: 11 April 2013.
Accepted for publication: 11 April 2013.

Rare Tumors 2013; 5:e24
doi:10.4081/rt.2013.e24

presence or absence of paternal genetic material. DNA from paraffin-embedded tissue, considered reference material, was compared to the patient's peripheral blood DNA using a FTA card. Following extraction of DNA from the formalin-fixed and paraffin wax embedded material,[3,4] all samples were quantified by NanoDrop, and a MiniFiler kit (Applied Biosystems) was used to amplify 10 ng DNA from each biopsy and FTA blood sample. Amplified products were then separated and detected using an ABI 3130 Genetic Analyzer (Applied Biosystems), with LIZ500 used to determine the length of the DNA fragments obtained. Electrophoresis results were analyzed using GeneMapper® ID v.3.2 (Applied Biosystems), and the genetic profiles of the biopsy and peripheral blood were compared. Both samples were found to be identical, indicating the absence of paternal DNA. For example, the population frequency of 8 short tandem repeats (STRs) confirmed that the blood sample and the biopsy sample shared the same origin, thereby confirming the presence of a non-gestational choriocarcinoma. The probability of randomly finding an individual with this genetic profile is estimated to be 1 in 410,788,655,648. Table 1 indicates the genetic profile of the patient and the population frequency of the profile. The clinical oncology team started the patient on BEP (Bleomicin, Ethoposide and Cisplatin) as a first-line treatment for an ovarian germ cell tumor. After the first cycle of BEP, the patient presented with an overall improvement in obstructive symptoms and vaginal bleeding, and was subsequently discharged to continue outpatient treatment.

During the treatment with four cycles of BEP, the patient was monitored monthly for serum levels of β-hCG, and a gradual decrease in tumor marker levels were also detected. Thirty days after the beginning of chemotherapy the β-hCG serum level was undetectable and we observed normalization of the LDH serum level. The patient developed grade 1 (common toxicity criteria, CTCAE 4.0) ototoxicity and grade 1 pneumonitis which was treated with prednisone.[5] The patient subsequently experienced a complete recovery, and a PET-CT was performed to evaluate the presence of residual tumor following chemotherapy. It was detected increased fluorodeoxyglucose (FDG) uptake in the periphery of the pelvic mass and pulmonary nodules (Figure 2).

The patient underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy two months after completion of chemotherapy. Furthermore, histological analysis showed an absence of residual tumor. Levels of β-hCG were also undetectable after surgery. Following the resection of a pelvic mass, the results of a PET-CT scan and undetectable serum levels of β-hCG indicated that the lung lesions were only composed of fibrosis and necrosis in the absence of viable tumor. A chest CT scan performed one month after surgery showed a significant decrease in the number of pulmonary lesions had occurred, and correspondingly, the patient did not manifest disease symptoms

Discussion

Choriocarcinoma of ovarian origin is extremely rare and can develop from an ectopic pregnancy or a rare and aggressive form of a germ cell tumor. Non-gestational choriocarci-

Table 1. Allelic polymorphism of 8 STRs (short tandem repeats) analyzed to human identification of paraffin-embedded tissue from tumor biopsy and blood. It was used allelic frequency of Caucasian-Applied Biosystems.

DNA *locus*	Alleles of FTA and biopsy		P	q	Population frequencies
D13S317	11	12	0.2980	0.3080	0.18357
D7S820	10	10	0.2722		0.08004
D2S1338	16	18	0.0473	0.0630	0.00596
D21S11	31	31.2	0.0716	0.0946	0.01355
D16S539	13	13	0.1676		0.03228
D18S51	14	18	0.1676	0.0774	0.02594
CSF1PO	10	10	0.2421		0.06412
FGA	20	24	0.1390	0.1375	0.03823

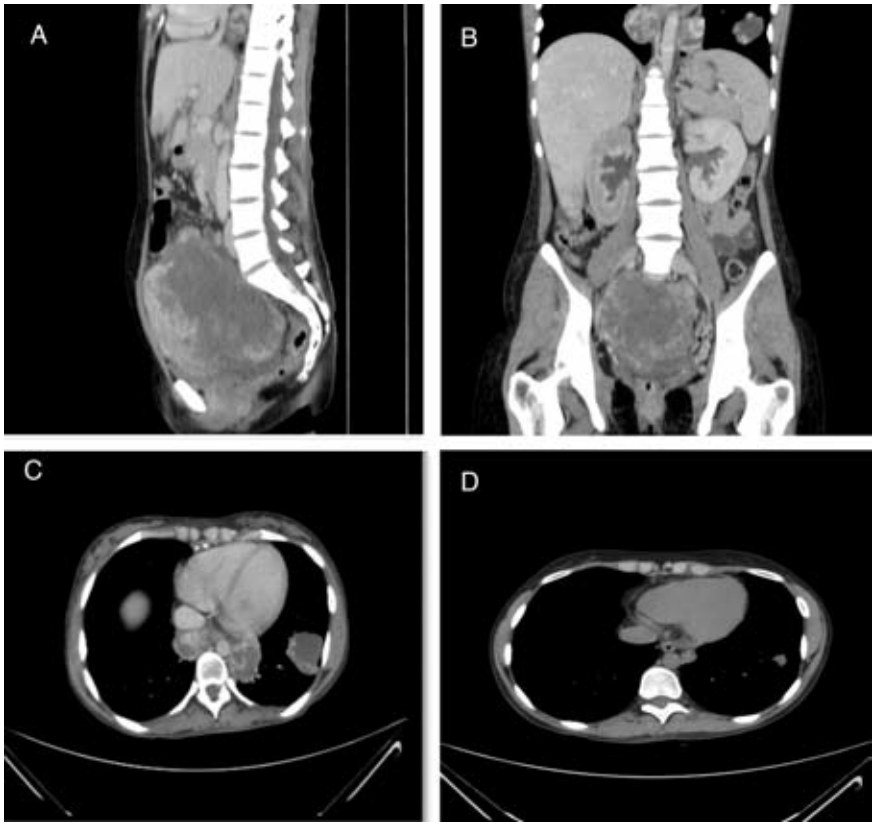

Figure 1. A) Extensive heterogeneous pelvic mass with peripheral enhancement and large necrotic component, measuring approximately 15.4×14.7×14.0 cm. It presents intimate contact with the bladder, ascending colon, and descending segments of the small intestine and the right iliopsoas and uterus. B) Coronal image showing bulky pelvic mass at diagnosis, showing pulmonary nodules, the largest in the left lung base measuring 3.7×3.6 cm. C) CT image during initial staging showing pulmonary metastases with heterogeneous contents and calcifications inside. D) Pulmonary metastasis after treatment with chemotherapy and surgery, measuring 10×10 mm, without the expression of serum with chemotherapy and surgery.

noma, is more common in women of childbearing age, and accounts for less than 1% of germ cell tumors of the ovary that are diagnosed.[6,7] Moreover, it often includes other malignant elements in its composition.

Differentiation between gestational choriocarcinoma and non-gestational choriocarcinoma can be very difficult since the clinical presentation and histology of these two diseases are identical and previous history of a normal pregnancy, spontaneous abortion or ectopic pregnancy has been used to make the diagnosis of gestational choriocarcinoma.[8,9] In addition, both tumors produce β-hCG, with an extremely fast growth pattern and metastatic disease to lung, liver and brain.[10] Histologically, both tumor types are characterized by abnormal throphoblastic hyperplasia and anaplasia, absence of chorionic vili, and the presence of hemorrhage and necrosis.[11] Due to the resulting atypical mitosis and high proliferation rate, patients with these diseases are subject to hemorrhage and ischemic necrosis.[12]

The mean serum level of β-hCG at diagnosis of a choriocarcinoma, either gestational or non-gestational, is 16,000 mIU/mL, with 597,000 mIU/mL being the highest value reported.[13,14] Correspondingly, periodic monitoring of β-hCG serum levels is extremely useful for evaluating a therapeutic response.[15]

In certain rare cases of gestational choriocarcinoma, tumors derived from placental tissue debris remaining from an endometrial abortion have been detected. The present case is intriguing due to the high level of serum β-hCG detected at diagnosis, and the previous history of abortion, these factors further complicate the differentiation of gestational versus non-gestational choriocarcinoma. Therefore, a polymorphic analysis of tumor DNA was necessary. Molecular techniques for the analysis of biopsies prepared as formalin-fixed or paraffin wax embedded tissues are available. In addition, if a mix-up in biopsies is suspected in a diagnostic laboratory, genetic identification of the tissues involved can establish the correct identities.[16,17] In the present case, the genetic profile of the biopsy was used to determine the origin of the tumor, and this was essential for determining the appropriate treatment for the patient.[8,18] For example, genetic analyses of the biopsy and peripheral blood of the patient revealed that both samples exhibited the same genetic profile, thereby excluding the possibility that an ectopic pregnancy had occurred. Furthermore, the probability of finding another genetic profile that would be identical to this patient in the entire population is 1 in 410,788,655,648. Accordingly, these results increase the reliability of the identification and treatment of the patient.

Despite the difficulty in differentiating gestational and non-gestational choriocarcinomas, these tumors types need to be distinguished, particularly since the latter is more resistant to chemotherapy, has a worse prognosis than a trophoblastic neoplasm, and its treatment approach involves surgery. In contrast, the initial treatment for primary choriocarcinoma of the ovary consists of cytoreductive surgery followed by adjuvant chemotherapy with BEP.[19,20] Moreover, patients with a residual mass, despite normalization of serum β-hCG levels, are also indicated for salvage surgery. These patients must be monitored for at least five years, and serum levels of -hCG should be assayed every three months for the first two years. Radiological monitoring should also be performed for two years from the start of normalized β-hCG levels.[21] In the present case, the lesion was first considered unresectable, and therefore, neoadjuvant chemotherapy with BEP was administered aiming a later surgical approach.

In contrast, treatment of gestational choriocarcinoma varies according to tumor stage. For example, in cases involving stage 1 disease, total hysterectomy is the treatment of choice. However, for patients of childbearing age, this treatment can be replaced with chemotherapy with methotrexate and leucovorin or dactinomycin.[21,22] Both approaches should be maintained for at least three cycles following the normalization of β-HCG serum levels. For patients with low-risk stage 2 and stage 3 tumors, they should receive the same regimen as patients with stage 1 tumors.[21,22] If these patients are refractory to the initial regimen, a MACIII (Methotrexate, Dactinomycin, and Cyclophosphamide) scheme must be indicated.[23]

Finally, patients at high risk for recurrence or metastatic disease should receive multidrug therapy with EMA-CO (Etoposide, Methotrexate, Dactinomycin, Cyclophosphamide, Vincristine, and Leucovorin) administered every two weeks, and this regimen should be maintained for three cycles following the normalization of serum levels of β-hCG.[24]

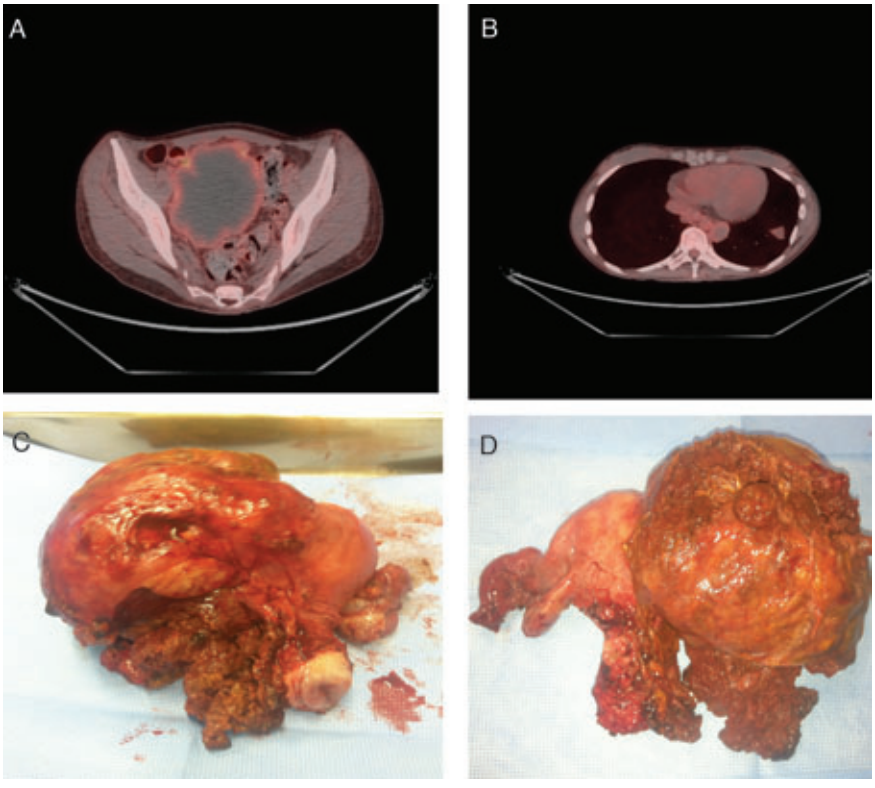

Figure 2. A) Pelvic mass with large central areas of necrosis/liquefaction after BEP (bleomicin, cisplatin, etoposide), 4 cycles, with increased glycolytic metabolism in the periphery (SUVmax: 3.5, SUV max fee: 4.0). B) Minimum increased glycolytic metabolism of multiple pulmonary nodules left (SUVmax: 1.3), and bilateral mediastinal limph nodes (SUVmax: 1.7), measuring up to 2.5 cm. C) Front view of hysterectomy specimen showing large mass attached to right annex, measuring 12 X 8.0 cm which anatomopathological examination showed no residual tumor. D) Posterior view of the specimen.

Conclusions

Non-gestational choriocarcinoma is extremely rare and is a very aggressive neo-

plasm. However, it has the potential to be cured with surgery followed by chemotherapy. Differentiation between this tumor type and gestational choriocarcinoma can be achieved with an analysis of tumor DNA to detect the presence of paternal DNA. Although this strategy is available, it is seldom utilized and is expensive, which may impact the selection of an appropriate treatment, and consequently, the prognosis of a patient.

References

1. Lv L, Yang K, Wu H, et al. Pure choriocarcinoma of the ovary: a case report. J Gynecol Oncol 2011;22:135.
2. Axe SR, Klein VR, Woodruff JD. Choriocarcinoma of the ovary. Obstet Gynecol 1985;66:111.
3. Coombs NJ, Gough AC, Primrose JN. Optimization of DNA and RNA extraction from archival formalin-fixed tissue. Nucleic Acids Res 1999;27:i.
4. Coura R, Prolla JC, Meurer L, Ashton-Prolla P. An alternative protocol for DNA extraction from formalin fixed and paraffin wax embedded tissue. J Clin Pathol BioTechniques 2005;58:894.
5. National Institutes of Health. National Cancer Institute. Cancer Therapy Evaluation Program. Common Terminology Criteria for Adverse Events, v4.0. Available from: http://ctep.cancer.gov/forms/CTCAEv4.pdf.
6. Smith HO, Berwick M, Verschraegen CF, et al. Incidence and survival rates for female malignant germ cell tumors. Obstet Gynecol 2006;107:1075.
7. Longo R, Battaglia F, Gattuso D, et al. Primary nongestational choriocarcinoma of the uterine cervix. J Clin Oncol 2011; 29:e301
8. Fisher RA, Newlands ES, Jeffreys AJ, et al. Gestational and nongestational trophoblastic tumors distinguished by DNA analysis. Cancer 1992;69:839.
9. Wheeler CA, Davis S, Degefu S, et al. Ovarian choriocarcinoma: a difficult diagnosis of an unusual tumor and a review of the hook effect. Obstet Gynecol 1990; 75:547.
10. El-Helw LM, Hancok BW. Treatment of metastatic gestational trophoblastic neoplasia. Lancet Oncol 2007;8:715.
11. Lurain JR, Gestational trophoblastic tumors. Semin Surg Oncol 1990;6:347.
12. Lurain JR, Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatiform mole. Am J Obstet Gynecol 2010;203:539.
13. Elliott MM, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the alpha- and beta-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 1997;7:15.
14. Cole LA, Dai D, Butler SA, et al. Gestational trophoblastic diseases: 1. Pathophysiology of hyperglycosylated hCG. Gynecol Oncol 2006;102:145.
15. Cole LA, Butler SA. Hyperglycosylated human chorionic gonadotropin and human chorionic gonadotropin free beta-subunit: tumor markers and tumor promoters. J Reprod Med 2008;53:499.
16. Horn LC, Edelmann J, Hanel C, et al. Identity testing in cervical carcinoma in case of suspected mix-up. Int J Gynecol Pathol 2000;19:387.
17. Orlandi F, Barucca A, Biagini G, et al. Molecular stability of DNA typing short tandem repeats in the mammary tree of patients with breast cancer. Diagn Mol Pathol 2002;11:41.
18. Hu T, Yang M, Zhu H, et al. Pure non-gestational ovarian choriocarcinoma in a 45,XO/46,XX SRY-negative true hermaphrodite. J Obstet Gynaecol Res 2011; 37:1900.
19. Williams S, Blessing JA, Liao SY, et al. Adjuvant therapy of ovarian germ cell tumors with cisplatin, etoposide, and bleomycin: a trial of the Gynecologic Oncology Group. J Clin Oncol 1994;12:701.
20. Gershenson DM. Management of ovarian germ cell tumors. J Clin Oncol 2007;25: 2938.
21. Berkowitz RS, Goldstein DP, Bernstein MR. Ten year's experience with methotrexate and folinic acid as primary therapy for gestational trophoblastic disease. Gynecol Oncol 1986;23:111.
22. Matsui H, Iitsuka Y, Seki K, et al. Comparison of chemotherapies with methotrexate, VP-16 and actinomycin-D in low-risk gestational throphoblastic disease. Remission rates and drug toxicities. Gynecol Obst Invest 1998;46:5.
23. Berkowits RS, Goldestein DP, Bernstein MR. Modified triple chemotherapy in the management of high-risk metastatic gestational trophoblastic tumors. Gynecol Oncol 1984;19:173.
24. Lurain JR, Singh DK, Schink JC. Primary treatment of metastatic high-risk gestational trophoblastic neoplasia with EMA-CO chemotherapy. J Reprod Med 2006;51: 767.