Therapy Related Myelodysplasia/Myeloproliferative Neoplasia-Unclassified with Acute Leukemic Transformation Following Paclitaxel and Carboplatin Based Chemotherapy in an Ovarian Cancer Patient

S. Vanajakshi · S. V. S. S. Prasad · S. S. Amina ·
E. Kavitha · K. Iravathy Goud · K. Kshitija

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Abstract Alkylating agents used in chemotherapy are mutagenic and have strong leukemogenic potential. The most serious long term complication of chemotherapy is the development of secondary disease, particularly hematological malignancy; they have rarely been reported in the context of ovarian cancer treatment. We describe quite a rare occurrence of a myelodysplastic/myeloproliferative neoplasm, unclassified (MDS/MPN-U) with acute leukemic transformation and multiple cytogenetic abnormalities not usually found together as JAK2 V617F mutation, 5q- and 7q-deletion, after exposure to paclitaxel and carboplatin based chemotherapy in a patient treated for ovarian cancer. We should be aware of such complication whose prognosis is really poor.

Introduction

Improvements in chemotherapy with introduction to paclitaxel and carboplatin have significantly increased the life expectancy of advanced ovarian cancers patients. Understanding the long term complication of these drugs is now

our priority. Therapy related MDS/MPN and therapy related acute leukemia is characterized by relatively long latency period, multilineage dysplasia, frequent chromosomal aberrations and an unfavourable response to treatment. These factors contribute to its poor prognosis. Therapy related secondary leukemia that follows treatment with alkylating agents is usually preceded by myelodysplasia (t-MDS). Development of secondary myeloid malignancy is a rare event among patients with a history of ovarian cancer [1]. Here we report an interesting *case of MDS/MPN-U with acute leukemic transformation in 3 years of treatment* with alkylating agent and antimicrotubule agent in case of ovarian cancer.

Case Report

A 66-year old postmenopausal woman came with a complaint of lower abdominal mass in July 2010, ultrasound detected left adnexal mass with elevated CA-125 levels (366 IU/mL/RR = < 21/U/mL). A total abdominal hysterectomy with bilateral salpingo-oophorectomy, pelvic lymph node dissection and omentectomy were performed. The peritoneal washing cytology was reported positive. The histopathologic diagnosis was poorly differentiated carcinoma. She was staged as FIGO stage IIIB and submitted to six courses of systemic chemotherapy with paclitaxel and carboplatin (total cumulative dose—1,200 and 2,700 mg respectively). Following completion of chemotherapy course in October 2010, her CA-125 levels were normalized with normal hematological parameters. Computed tomography and Magnetic resonance imaging showed no residual diseases. Based on these findings she achieved in remission and was followed with routine surveillance (Tables 1, 2).

S. Vanajakshi and K. Kshitija have contributed equally to work.

S. Vanajakshi (✉) · S. S. Amina · K. Kshitija
Department of Haematology and Clinical Pathology, Apollo
Hospitals, Jubilee Hills, Hyderabad 500096, India
e-mail: vanajakshi_s@apollohospitals.com

S. V. S. S. Prasad
Department of Oncology, Apollo Hospitals, Hyderabad, India

E. Kavitha · K. Iravathy Goud
Department of Cytogenetics & Molecular Biology, Apollo
Hospitals, Hyderabad, India

Table 1 Comparison of haematological parameters before surgery, during and after chemotherapy

Haematological parameters	Before chemotherapy	Chemotherapy cycles						1 month after chemotherapy
		I	II	III	IV	V	VI	
Haemoglobin (g/dL)	11.6	10.8	9.9	9.5	8.8	9.3	9.7	10.1
Total leucocyte (count/mm ³)	8,800	3,400	4,700	4,400	3,300	3,500	5,500	4,800
Platelet count $\times 10^3$ (mm ³)	462	254	402	377	250	335	260	389
Neutrophil (%)	52	37	39	28	24	33	35	37
Lymphocyte (%)	38	52	54	65	67	60	60	55
Eosinophil (%)	03	01	00	02	00	00	00	03
Monocyte (%)	07	10	07	05	09	07	05	08

Table 2 CA125 levels before and after chemotherapy

Chemotherapy	CA 125 (U/L)
Before	366
After	5.8

Following 27 months of disease free survival, she was admitted on February 2013 with anemia and received two units of blood transfusion. Her peripheral blood count status was as follows: hemoglobin 9.2 g/dL, total leukocyte count $17.8 \times 10^9/L$ (8 % blasts, 28 % myelocyte, 6 % metamyelocyte, 8 % neutrophils, 38 % lymphocytes, 8 % eosinophils, 4 % monocytes and 2 % basophils) and platelets $196 \times 10^9/L$. Peripheral smear shows macroovalocytes, 8 % blasts and left shift with hypogranular metamyelocytes and macrohypogranular dyspoietic platelets (Fig. 1). Bone marrow aspiration and biopsy revealed hypercellular marrow. The erythropoiesis is normoblastic and hyperplastic. The granulopoiesis is hyperplastic with 9 % blasts. The megakaryopoiesis is hyperplastic and dyspoietic with prominence of hypolobated megakaryocytes (Figs. 2, 3). Reticulin stain on marrow biopsy revealed grade 3 on the scale of 0–3 (Fig. 3) whereas Perl's stain revealed normal iron stores with no ring sideroblast.

FISH analyses on bone marrow aspirate showed 5q31 and 7q31 deletion (Fig. 4) whereas a mutation of JAK2V617F was present by qualitative PCR analysis.

Based on these findings a diagnosis of therapy related MDS/MPN-U was rendered. On follow up, her peripheral blood picture constantly showed leucoerythroblastic picture for 5 months. On 6th month, her counts were as follows: hemoglobin 7.38 g/dL, total leucocyte count $100.3 \times 10^9/L$ (19 % blasts, 6 % promyelocyte, 20 % myelocyte, 6 % metamyelocyte, 6 % bandforms, 20 % neutrophils, 11 % lymphocytes, 5 % eosinophils and 7 % monocytes) and platelets $196 \times 10^9/L$ (Fig. 5).

Myeloperoxidase cytochemical staining on peripheral blood smear showed weak positivity in 12 % blasts whereas Periodic acid schiff stain was negative in all the

blasts (Fig. 5). A diagnosis of therapy related MDS/MPN-U transforming into acute leukaemia was given. Patient refused for further treatment and in within 2 weeks time her total counts became $402.5 \times 10^9/L$ with 80 % blasts. Immunophenotyping by flow cytometry on peripheral blood revealed CD45/side scatter gated population of 88 % blasts (dim to moderate expression). These blasts showed myeloid lineage markers as follows: 66 % CD13 (dim to moderate expression), 27 % CD15 (dim to moderate expression), 98 % CD33 (dim to moderate expression), 21 % CD117 (dim expression) and 54 % cMPO (dim expression). These blasts also showed monocyte lineage markers as follows: 26 % CD14 (dim expression) and 57 % CD64 (dim expression). An aberrant expression of 37 % CD56 (dim expression) was also noted (Fig. 6).

G-banding analysis of her peripheral blood cells demonstrated 53 XX, +8, +12, +16, +18, +19, +2mar(10)/46, XX(15) (Fig. 7).

Informed written consent was obtained from the patient and is approved by institutional ethical committee.

Molecular studies for the most frequent AML-related alterations (CBF β /MYH11, DEK/CAN, NPM1, FLT3, and RUNX1/ETO) showed no abnormalities. Her condition deteriorated and she eventually died due to cardiac arrest and pulmonary cerebral leucocytostasis within a month after her initial diagnosis of acute leukaemia. The time course of events is depicted in (Table 3).

Discussion

Ovarian cancer is the most common gynaecological malignancy and ranks fifth as a cause of death in women. Fortunately, ovarian carcinomas are sensitive to chemotherapy and most patients with advanced stage disease receive chemotherapy that increases their survival and disease free interval and improves quality of life. At present the chemotherapeutic drugs of choice for ovarian cancer are paclitaxel and carboplatin. Before the introduction of these drugs, the response rate was only 40 % in

advanced ovarian cancer. Medium survival was 1 year and only small percentage of patients survive for 5 years [2]. With paclitaxel and platinum based therapy the response rate increased to 60–70 % with survival rate of 20–30 % after 5 years [3]. Paclitaxel is an antimicrotubule agent. It binds β -tubulin, but only when the monomer is incorporated into a microtubule. The binding site is on the inner face of the polymer, and the drug can bind the length of the polymer. Drug binding stabilises the structure of the polymer by inducing a conformational change, which enhances the affinity of the interaction between tubulin molecules. Stabilisation of microtubules by paclitaxel binding prevents normal formation of mitotic spindles. On entry into mitosis, chromosomes can attach paclitaxel-stabilised microtubules; however, the lack of microtubule dynamics means that tension is not produced across sister chromatids, and prevents correct chromosome bi-orientation. This leads to chronic activation of the spindle-assembly checkpoint (SAC), which in turn leads to mitotic arrest [4, 5]. The platinum complexes react *in vivo* binding to and causing cross linking of DNA which ultimately triggers apoptosis. Thus these two are unlikely

incorporated into DNA to interfere with DNA repair mechanisms. The carboplatin and/or paclitaxel induced event may represent evidence of genomic damage to the bone marrow that may be disposed to multiple genetic changes [6, 7]. The synergistic effect of paclitaxel and carboplatin yields higher sensitivity with overall response rate of 75 %. Additionally the combination is tolerable with fewer side effects and only produces mild myelosuppression. The dose limiting toxicities of combined carboplatin and paclitaxel chemotherapy are nephrotoxicity, neurotoxicity and myelotoxicity (anemia, leucopenia and thrombocytopenia). However as more patients survive longer, the delayed combination of these drugs including the development of a potentially life threatening secondary malignancy must be considered. The secondary leukaemias resulting from alkylating agents have complex karyotypes that confer a poor prognosis [8]. Therapy-related myeloid neoplasms (t-MN) account for approximately 10–20 percent of all cases of AML, MDS and MDS/MPN. The diagnosis of therapy-related myeloid leukemia (t-MDS/t-AML) identifies a group of high-risk patients with multiple and varied poor prognostic features. The interval between

Fig. 1 Peripheral smear shows MDS with trilineage dysplasia. **a** Macroovalocytes with macrohypogranular dyspoietic platelets. **b** Blast and hypogranular metamyelocyte (Giemsa stain, $\times 100$)

Fig. 2 Bone marrow aspiration cytology shows MDS with trilineage dysplasia. **a** Binucleate normoblast. **b** Pelgeroid neutrophil with hypogranular myelocyte. **c** Micromegakaryocyte with hypolobated partially detached nucleus, (giemsa stain, $\times 100$)

Fig. 3 Bone marrow biopsy shows MDS with trilineage dysplasia. **a** Hyperplastic and dyspoietic erythropoiesis, hyperplastic granulopoiesis with prominence of blasts, hyperplastic megakaryocytes with

hypolobated micromegakaryocytes (H and E, ×400). **b** Hypolobated micromegakaryocytes, (PAS, ×400). **c** Grade 3 on scale of 0–3 (reticulin, ×400)

Fig. 4 **a** Interphase cell showing single spec orange signal 5q31 (EGR1) deletion (5q–), **b** interphase cell showing single spec orange signal 7q31 (D7S522) deletion (7q–)

Fig. 5 Peripheral smear shows acute leukaemic transformation. **a** Immature leucocytes, with prominence of blasts (Giemsa stain, ×100). **b** Some of the blasts show myeloperoxidase, granules (MPO, ×100). **c** Blasts show negative staining on PAS (PAS, ×100)

the alkylating agent exposure and the development of t-AML is 5–7 years and majority of cases are preceded by myelodysplasia whereas 20 % arise denova. Our case developed t-AML by 3 years of exposure to alkylating agent and it was preceded by not only myelodysplasia but also myeloproliferative neoplasia. The median duration of survival is 8 months, and the 5-year survival rate after diagnosis t-MDS/t-AML is 10 % [9]. Only few cases of the

secondary diseases, particularly MDS and acute myeloid leukemia following ovarian cancer therapy are reported in the literature and in those cases only carboplatin and paclitaxel were used as primary chemotherapy [10]. Most of the secondary leukaemia belong to M4 variety followed by M5, M6 and M7 [10]. Our case had AML M4 with 5q31 and 7q31 deletion, mutation of JAK2V617F along with trisomies 8, 12, 16, 18, 19. The cases stated (Table 4)

Fig. 6 Flow cytometric analysis of diagnostic markers in acute myelomonocytic leukaemia (M4). **a** Blasts are identified by CD45/SSC gating, showing 88.6 % expression. **b** Myeloid leukaemia with co expression of CD33 and CD15 [27.8 %]. **c** 54 % cMPO expression. **d** 66.4 % CD13 expression Monocytic leukaemia is identified by 26.1 % CD14 expression. **e** 57.6 % of CD64 expression. **f** Blasts also show aberrant expression of CD 56 [36 %] and 71.3 % CD38 non lineage marker expression

Fig. 7 Karyotype. **a** Metaphase showing 53 chromosomes. **b** Trisomies of chromosomes 8, 12, 16, 18, 19 and marker chromosomes (53 XX, +8, +12, +16, +18, +19, +2mar)

Table 3 Time line of events from initial diagnosis of ovarian cancer and following carboplatin- and paclitaxel-based chemotherapy to the onset of myelodysplastic syndrome/myeloproliferative neoplasia, unclassified till onset of AML-M4

Ovarian cancer stage IIIB			Onset of MDS/MPN-U	Onset of AML-M4	Expired
↓			↓	↓	↓
July 2010	2011	2012	February 2013	6 August 2013	22 August 2013

Table 4 Journal review of the secondary AML cases following exposure to paclitaxel [3]

Author	Age	Primary tumour	Latency (months)	Preceding disease	French American British class	Cytogenetic abnormality	Prognostic group
Seymour et al. [6]	57	Ovarian adenocarcinoma	10	No	M4	Inv(16)(p13q22)	Favorable
Seymour et al. [6]	53	Neuroendocrine ovarian carcinoma	8	No	M4	Inv(16)(p13q22)	Favorable
See et al. [9]	52	Ovarian carcinoma	23	MDS	M6	45XX, t(1,5)(q25;35) Del(4)(q21q26), Ins(7)(p15;?),del(12) (p12), -13,-17,add(19) (p13.3),add22(q13)	Poor
Tasaka et al. [7]	43	Ovarian carcinoma	12	No	M5b	47XX, +8, t(8;16)(p11;p13)	Poor
Dissing et al. [5]	43	Ovarian carcinoma	61	NS	M4	Inv(16)(p13q22)	Favorable
Dissing et al. [5]	51	Leiomyosarcoma	33	NS	M4	Inv(16)(p13q22)	Favorable
Yeasmin et al. [3]	73	Ovarian adenocarcinoma	25	MDS	M7	46XX, del(5;19)(p10;q10),-6,del(7)(q?),add(12) (p11.2), -13, -17, +19	Poor
Current	66	Poorly differentiated ovarian carcinoma	36	MDS/MPN-U	M4	5q-,7q-, 53 XX,	

+8, +12, +16, +18, +19, +2mar JAK2V617F mutation

shows latency between t-AML and prior treatment with paclitaxel in ovarian cancers.

In conclusion, our patient had several predisposing factors like older age, advanced stage ovarian cancer, poorly differentiated carcinoma, exposure to alkylating agent, paclitaxel, myelodysplasia with myeloproliferative neoplasm and multiple chromosomal abnormalities for the transformation to t-AML. The interesting fact of our case is the *development of t-MDS/MPN-U with 5q and 7q deletion along with JAK2 mutation progressing to AML M4 with abnormal karyotyping in 3 years of exposure to chemotherapeutic agents in a poorly differentiated ovarian cancer.*

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