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Case report

Leukocytosis due to markedly elevated granulocyte-colony stimulating factor levels in a patient with endometrial cancer: Case report and literature review

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1. Introduction

Extreme leukocytosis with peripheral blood white cell counts above 40 or 50 × 10^9/L has been reported in many solid tumors (Granger and Kontoyiannis, 2009). Up to 10% of cases of extreme leukocytosis (≥40 × 10^9/L) in patients with solid tumors can be attributed to a paraneoplastic leukemoid reaction (PLR) (Granger and Kontoyiannis, 2009). PLR can be caused by cytokine production by the tumor, such as granulocyte-colony-stimulating factor (GCSF), granulocyte-macrophage-colony-stimulating factor (GMCSF), interleukin-1 alpha, and interleukin-6 (Lee et al., 1989). Elevations in cytokines induce an autocrine growth cycle, which stimulates tumor growth, and may contribute to the poor overall prognosis seen in patients with PLR (Lee et al., 1989).

Tumor production of GCSF in gynecologic malignancy is rare (Ahn et al., 2005; Connor, 2006; Granger and Kontoyiannis, 2009; Hada et al., 2004; Kyo et al., 2000; Mabuchi et al., 2010; Matsumoto et al., 2010; Mikami et al., 2005; Nakayama et al., 2012; Nasu et al., 2004; Sudo et al., 1996; Watanabe et al., 2000; Yabuta et al., 2010; Yamamoto et al., 2013). In this case report, we describe a patient with endometrial cancer who developed unexplained leukocytosis during treatment of recurrent disease. Marked elevation in serum GCSF was noted but tumor stained negative for GCSF. We review the potential mechanisms of elevated serum GCSF in advanced cancer and the available literature on GCSF secreting gynecologic tumors.

2. Case report

The patient is a 65-year-old G2P2 who originally presented in April 2012 with vaginal spotting. Pap smear by her primary care physician showed adenocarcinoma and she was referred to our Gynecologic Oncology clinic at a large tertiary-care institution.

At the time of initial consultation, physical examination was unremarkable. Her body weight was 81 kg and body mass index 36 kg/m^2. She had no evidence of cervical, supraclavicular, or inguinal adenopathy. Her abdominal exam was without palpable mass. Genitourinary exam revealed a normal appearing cervix. The patient underwent colposcopy, endocervical curettage and endometrial biopsy. Her endometrial biopsy revealed a FIGO grade 2 endometrioid adenocarcinoma of the uterus. The patient consented to tumor banking for medical research under UNC IRB 90-0573.

In June 2012, she underwent surgical staging with robotic-assisted total laparoscopic hysterectomy with bilateral salpingoophorectomy, and bilateral pelvic and para-aortic lymphadenectomy. Final surgical pathology revealed a mixed serous (40%) and endometrioid (60%) tumor, International Federation of Gynecology and Obstetrics (FIGO) grade 3, with an isolated positive right para-aortic lymph node; overall FIGO Stage IIIC2. She was dispositioned to platinum/taxane chemotherapy with extended field pelvic radiation and high-dose rate brachytherapy in a sandwich fashion. She completed treatment in February 2013. Her adjuvant treatment course was complicated by grade 3 neutropenia following her fifth cycle of chemotherapy, but was otherwise well tolerated.

In November 2013, she was found to have a vaginal lesion on surveillance exam. Subsequent computer tomography (CT) scan showed an isolated vaginal recurrence invading the bladder. The patient was offered exenterative procedure versus chemotherapy. She opted for chemotherapy and was treated with liposomal doxorubicin and carboplatin. There was initial partial response to chemotherapy, but her treatment course was complicated by myelosuppression, requiring multiple blood transfusions for anemia and treatment delays for thrombocytopenia. In August 2014, after 9 cycles of liposomal doxorubicin

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with carboplatin, she was switched to Megace due to her poor tolerance of chemotherapy and overall stable disease.

In December 2014, she developed a leukocytosis of unknown etiology. Her white blood cell (WBC) count was 21.8 × 10^9/L on routine lab work (Fig. 1). She was evaluated and found to be without evidence of infection. She was continued on Megace treatment at this time. On CT imaging she had progression of her bladder mass without distant metastatic disease. Urology performed a biopsy of the bladder mass, which returned consistent with known recurrent uterine cancer.

She remained off cytotoxic chemotherapy due to persistent profound anemia requiring transfusion. Hematology was consulted in March 2015. At this time her WBC were 30.6 × 10^9/L and she was requiring numerous transfusions for profound anemia. A work up was obtained with normal B12 and folate levels, adequate iron stores, and no evidence of hemolysis. Review of her peripheral smear showed numerous neutrophils with a left shift and evidence of toxic granulation, Döhle bodies, and occasional nucleated red cells and echinocytes. The WBC differential showed profound neutrophilia, however, there was no significant increase in other white cell lineages and no evidence of blasts. Ultimately, hematology felt her anemia and thrombocytopenia were due to therapy-related myelodysplastic syndrome and recommended supportive treatment with erythropoietin and transfusions; she did not receive any GSCF therapy. Her leukocytosis at that time was attributed to a malignant leukemoid reaction. A bone marrow biopsy was discussed but not performed as it was felt that it would not alter her management in the setting of her advanced endometrial cancer. In May 2015, hematology consultation was again obtained for persistent severe anemia and thrombocytopenia with worsening leukocytosis (WBC 51.4 × 10^9/L) and a potential diagnosis of paraneoplastic GCSF production was raised. Peripheral smear and differential remained similar at this time without concern for acute or chronic leukemia. Serum GCSF was measured and found to be 2264.5 pg/mL (normal range 0.0–39.1 pg/mL). This value was calculated via an enzyme-linked immunosorbent assay through a commercially available, validated kit at the University of Minnesota Outreach Laboratories. This assay is not FDA approved at this time.

Ultimately, due to severe persistent anemia and thrombocytopenia requiring multiple chronic blood and platelet transfusions, the patient was deemed not to be a candidate for further cytotoxic chemotherapy or surgery and was placed on hospice care. She expired in July 2015, eight months following development of leukocytosis.

Given the elevated GCSF levels, the patient's initial tumor and recurrent tumor were stained using a monoclonal anti-GCSF antibody obtained commercially (Santa Cruz Labs, CA), after obtaining consent from the patient's next of kin. Formalin-fixed paraffin-embedded (FFPE) sections of the bladder biopsy with recurrent tumor were immunostained in the Bond fully-automated slide staining system (Leica Microsystems). Slides were dewaxed in Bond Dewax solution (AR9222) and hydrated in Bond Wash solution (AR9590). Heat induced antigen retrieval was performed for 20 min in Bond-Epitope Retrieval solution2 pH-9.0 (AR9640). The antigen retrieval was followed with 5 min Bond peroxide blocking (DS9800) and 10 min Bond protein blocking (PV6122) steps. After pretreatment goat polyclonal anti-G-CSF antibody (1:100) was applied for 1 h (sc-1318, SCBT, TX). Detection was performed using Bond Intense R Detection System (DS9263) supplemented with ImmPRESS HRP Anti-Goat Ig (Peroxidase) Polymer (#MP-704-15, Vector Labs. CA). Stained slides were dehydrated and cover-slipped. Positive and negative controls (no primary antibody) were included for each run. Both the original tumor and the recurrent tumor (biopsied at the time of leukocytosis) were negative for GSCF, suggesting the tumor was not producing the GCSF measured in her serum.

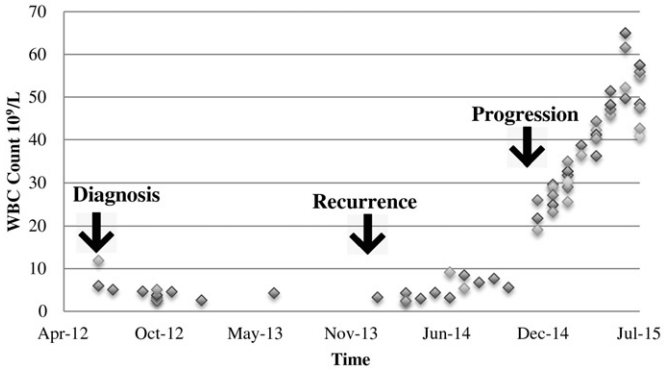

Fig. 1. White blood cell count trended from diagnosis.

3. Discussion

Paraneoplastic production of GCSF was first reported in 1977 in a patient with lung cancer (Asano et al., 1977). It has since been reported in the literature in many tumor types. One large review of 3770 solid tumor patients found that 20% (n = 758) of patients had laboratory values consistent with extreme leukocytosis (WBC > 40 × 10^9/L). Of these patients, 77 patients (10%) had extreme leukocytosis attributable to PLR based on exclusion of other causes. In this cohort, resolution of leukocytosis was seen with successful cancer treatment in 10% of patients, but rapid progression and death within 12 weeks was the outcome in 70% of these patients (Granger and Kontoyiannis, 2009). In gynecologic malignancies, there have been a total of 18 cases reported of leukocytosis and elevated GCSF in addition to the 4 women in the above-mentioned review. Overall, fourteen women had cervical cancer (Ahn et al., 2005; Connor, 2006; Granger and Kontoyiannis, 2009; Kyo et al., 2000; Mabuchi et al., 2010; Matsumoto et al., 2010; Nasu et al., 2004; Watanabe et al., 2000; Yabuta et al., 2010), five had uterine cancer (Granger and Kontoyiannis, 2009; Hada et al., 2004; Nakayama et al., 2012; Yamamoto et al., 2013), and three had peritoneal/ovarian cancer (Granger and Kontoyiannis, 2009; Mikami et al., 2005; Sudo et al., 1996). These cases are summarized in Table 1.

While it is postulated that PLR is due to GCSF production by the tumor, endothelial cells and immune cells, such as macrophages and monocytes, can also produce GCSF. Profound immune activation as a reaction to tumor could also cause significantly elevated GCSF levels. GCSF serum levels in normal individuals range between 0 pg/mL and 10–39 pg/mL depending on the reference laboratory. The most common reason for elevations in the serum GCSF level is an infectious etiology, but large tumor burden can also be causative. In PLR, GCSF levels have been reported to be between 33 pg/mL and 1500 pg/mL. Our patient's level of 2264.5 pg/mL is much higher than previously reported levels and was not associated with tumor GCSF secretion. The source of GCSF production in our patient is unclear given the negative tumor staining. It is possible that rather than tumor secretion of GCSF, there was a profound immune response to the patient's rapidly progressing tumor.

Nearly all reported patients with GCSF secreting tumors have had rapid progression to death. It is postulated that this poor prognosis is secondary to autocrine tumor stimulation by GCSF and other cytokines (Lee et al., 1989). Much like other authors have presented in cases of GCSF secreting tumors, our patient expired within 8 months of developing her leukocytosis. While she was unable to undergo aggressive chemotherapy due to myelosuppression, other patients have had favorable results and improvement in leukocytosis with aggressive chemotherapy (Hada et al., 2004). Diagnosing a GCSF producing tumor or elevated GCSF level early in a patient's treatment course may allow a provider to recommend attempting aggressive chemotherapy with a goal of improved clinical outcome. Further, providers should be aware of the poor prognosis associated with elevated GCSF levels and consider transitioning patients to palliative care if aggressive treatment would be poorly tolerated.

Most prior reports of GCSF secreting tumors have described leukocytosis at the time of cancer diagnosis with fluctuations in the WBC with

Table 1
Cases of elevated GCSF in gynecologic malignancy.

Tumor site	Author	Number of cases	Clinical outcome	Histology	Leukocytosis on presentation	Serum GCSF level (normal range)
Cervix	Matsumoto et al.	4[b]	All cases recurred within 6 months and died within 15 months	Squamous	Yes	248, 106, 875, and 50.3 pg/mL (<18.1 pg/mL)
	Kyo et al.	1[b]	Rapid progression to death in 11 months	Squamous	Yes	197 pg/mL (<10.0 pg/mL)
	Nasu et al.	1[b]	Responded to treatment, NED 8 months after treatment	Squamous	Yes	195 pg/mL (5.78–27.5 pg/mL)
	Conner	1[b]	Rapid progression to death 10 weeks after initial treatment	Carcinosarcoma	Yes	1500 pg/mL (<10 pg/mL)
	Ahn et al.	1[b]	Rapid progression and death during initial treatment	Squamous	Yes	Not measured
	Watanabe et al.	1[b]	Rapid progression to death within 12 months	Small cell	Yes	269 pg/mL (<30 pg/mL)
	Yabuta et al.	2[b]	Death within 12 months of surgery	Squamous	Yes	125.2 pg/mL 811.4 pg/mL (2.6–32.0 pg/mL)
	Mabuchi et al.	2[b]	Both with rapid progression to death in 6 months	Adenocarcinoma	Yes	148 pg/mL[c] (<18.1 pg/mL)
	Granger et al.	1	Not documented	Not documented	Not documented	Not documented
Uterus	Nakayama et al.	1[b]	Death within 2 months of recurrence	Leiomyosarcoma	Unknown, authors treated for recurrence	33 pg/mL (<18.1 pg/mL)
	Yamamoto et al.	1[b]	Rapid progression to death in 1 month	Undifferentiated endometrial carcinoma	Yes	305 pg/mL (<18.1 pg/mL)
	Hada et al.	1[b]	NED at time of report	Endometrioid, poorly differentiated	Yes	284 pg/mL (<30 pg/mL)
	Granger et al.	2	Not documented	Not documented	Not documented	Not documented
	This report (Clark et al.)	1[d]	Progression to death 8 months after leukocytosis	Endometrioid	No, developed with recurrence	2264.5 pg/mL
Ovary, fallopian tube, peritoneum	Mikami et al.	1[b]	Death within 5 months of diagnosis	Serous peritoneal	Yes	Unknown[a]
	Sudo et al.	1[b]	Rapid progression to death during induction chemotherapy	Undifferentiated ovarian	Yes	1200 pg/mL (<39.1 pg/mL)
	Granger et al.	1	Not documented	Not documented	Not documented	Not documented

[a] The authors did not report the exact values, but Fig. 1 of the report shows values of approximately 50–300 pg/mL).
[b] Tumor tissue stained for GCSF and found to be positive.
[c] One patient declined blood draw.
[d] Tumor tissue tested for GCSF staining, but found to be negative.

treatment response. Our patient developed leukocytosis and elevated GCSF levels during her recurrence. This atypical presentation of GCSF elevation further supports the theory that progression and immune response were the cause of elevations in GCSF in this patient, rather than tumor secretion of GCSF as seen in other reports.

4. Conclusions

Providers should maintain suspicion for paraneoplastic leukemoid reaction in solid tumor patients with unexplained marked leukocytosis, such as peripheral blood white cell counts of $>40 \times 10^9$/L. There are currently no treatment modalities available to disrupt the proposed autocrine cycle leading to rapid tumor progression, but aggressive chemotherapy may be beneficial. Providers should counsel patients and families diagnosed with PLR regarding the overall poor prognosis associated with this state.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Acknowledgement

Informed consent was obtained from the patient's next of kin for publication of this case report. A copy of the consent is available for review by the Editor-in-Chief of this journal on request.

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