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Paraneoplastic Erythrocytosis of Colon Cancer, with Serum Erythropoietin within the Normal Reference Range

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Conflict of interest: None declared

Patient: Female, 75
Final Diagnosis: Erythropoietin-secreting colon cancer
Symptoms: None
Medication: —
Clinical Procedure: Immunohistochemistry
Specialty: Hematology

Objective: Rare disease
Background: Paraneoplastic erythrocytosis can be brought on by ectopic erythropoietin production usually in kidney, brain, and liver tumor with increase of serum erythropoietin level. We report here a paraneoplastic erythrocytosis of colon cancer with serum erythropoietin within the normal reference, which required an immunohistologic test for erythropoietin-antibody to be diagnosed.

Case Report: Our case report was of a 75-year-old woman with erythrocytosis. Her hemoglobin and serum erythropoietin levels were 191 g/dL and 12.6 IU/L (reference range, 9.1–32.8), respectively. Colonoscopy revealed an advanced sigmoid colon tumor 20 mm in diameter. She underwent colectomy, and immunohistochemical examination showed the colon adenocarcinoma was focally positive for erythropoietin-antibody. One month after the surgery, her hemoglobin level decreased to 117 g/L.

Conclusions: Colon cancer can cause paraneoplastic erythrocytosis, and it is important to consider not simply the absolute serum erythropoietin level but also the serum erythropoietin level relative to simultaneously measured hemoglobin level. We should include paraneoplastic erythrocytosis as a differential diagnosis in cases of high hemoglobin level unexplained by other diseases.

MeSH Keywords: Colorectal Neoplasms • Immunohistochemistry • Paraneoplastic Syndromes • Polycythemia

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Background

Primary erythrocytosis is the autonomous proliferation of red cell progenitor and precursor cells in the bone marrow; secondary erythrocytosis is paraneoplastic erythrocytosis. It occurs when a factor outside the bone marrow stimulates erythropoiesis [1]. Lack of the primary erythrocytosis features should be checked to diagnose paraneoplastic erythrocytosis, such as *JAK2* mutation and pancytosis. If primary erythrocytosis is suspected, bone marrow biopsy and stem cell culture are considered [1]. Ectopic erythropoietin (EPO) causes paraneoplastic erythrocytosis organizing usually from renal cell carcinoma, cerebral hemangioblastoma, and hepatoma [2,3]. EPO-secreting tumor is diagnosed by return of originally increased hemoglobin and serum EPO to normal level after tumor resection [2]. However, EPO-secreting colorectal cancer has been reported only rarely, and an EPO-secreting tumor with serum EPO within the normal reference range, never so far.

We here report paraneoplastic erythrocytosis of colon cancer, with serum EPO within the normal reference range. Immunohistochemical test for EPO-antibody was essential in diagnosing this condition.

Case Report

The case was a 75-year-old Japanese woman referred to our hospital because of incidentally found erythrocytosis. She had smoked 1 pack of cigarettes per day for over 50 years and had no drinking habit. Her hemoglobin level and hematocrit were 191 g/L (reference range, 108–149) and 0.556 (reference range, 0.356–0.454), respectively, without dehydration. Leukocyte and platelet counts were 5.1×10^9 /L (reference range, 3.3–9.9) and 166×10^9 /L (reference range, 148–361), respectively. Neutrophil alkaline phosphatase score was 162 (reference range, 169.5–335.0). Serum vitamin B12 level was not increased. Electrocardiogram and chest X-ray findings were normal. Arterial oxygen saturation was not decreased. The patient had no splenomegaly or chronic kidney disease and tested negative for *JAK2* mutation. Serum EPO level by radioimmunoassay was 12.6 IU/L (reference range, 9.1–32.8). She received multiple blood sampling, and hemoglobin, hematocrit, and serum EPO level remained stable. Magnetic resonance imaging ruled out cerebellar tumor. Esophagogastroduodenoscopy showed no tumor, and colonoscopy revealed an advanced sigmoid colon tumor 20 mm in diameter. Computed tomography showed no evidence of metastases, no tumor of liver, uterus, or kidney, and no hydronephrosis. Tumor markers, including carcinoembryonic antigen and carbohydrate antigen 19-9, were normal. She underwent laparoscopic sigmoid colon resection with minimal bleeding. Pathologic examination showed well-differentiated tubular adenocarcinoma infiltrating the proper

Figure 1. Histologic findings of the sigmoid colon specimen ($\times 100$). Hematoxylin and eosin staining of the tumor shows well-differentiated tubular adenocarcinoma.

muscular layer with a regional nodal metastasis and no residual tumor (Figure 1).

We performed immunohistochemical assays on formalin-fixed, paraffin-embedded sections with both the benign and the malignant tissue. Slides were stained by BOND-MAX (Leica Biosystems, Nussloch, Germany), and the primary antibody was mouse monoclonal to EPO (EPO2 1:400 dilution; Abcam, Cambridge, UK). Antigen retrieval was performed by BOND-MAX heating for 20 minutes in Bond Epitope Retrieval Solution 2 (Leica Biosystems) and Bond Polymer Refine Detection (Leica Biosystems Newcastle, Newcastle, UK). The colon adenocarcinoma was focally positive for EPO-antibody, and the patient's benign colonic mucosa was negative (Figure 2). One month after the colectomy, her hemoglobin level decreased to 117 g/L and serum EPO level was 36.6 IU/L. We did not perform bone marrow examination because her hemoglobin level remained stable with continuous smoking.

Discussion

This case highlighted 2 important clinical issues. Colon cancer can cause paraneoplastic erythrocytosis. It is important to consider not simply the absolute serum EPO level, but always the serum EPO level relative to simultaneously measured hemoglobin level.

First, colon cancer can cause paraneoplastic erythrocytosis. This is a rare case of EPO-secreting colon cancer. Kidney tumor accounted for more than a half (53%) of paraneoplastic erythrocytosis cases and the rest was caused by liver (19%), brain (15%), uterus, adrenal, ovary, lung, and thymus tumors [3]. Only 1 such case of colon cancer had been reported as paraneoplastic erythrocytosis: actually an EPO-secreting liver metastasis [4].

Figure 2. Immunohistochemical findings of mouse monoclonal antibodies to erythropoietin (×100). The colon adenocarcinoma (A) and the patient's benign colonic mucosa (B) were focally positive and negative, respectively.

EPO gene expression is primarily modulated by tissue hypoxia mediated by hypoxia-induced inducible factor-1 [5–7]. The production of EPO in organs other than kidney and fetal liver has been reported, including astrocytes and human female reproductive organs [8,9]. Additionally, several carcinoma cells were shown to express high levels of EPO protein [10,11]. Thus, it is understandable that kidney, liver, brain, uterus, and ovary tumors ectopically produce EPO. Colon adenocarcinoma had not been considered an EPO-secreting tumor; however, the uniform increase in the expression of EPO along the colonic neoplastic sequence was recently reported [12]. The patient did not have hypoxia, cardiopulmonary disease, or signs of primary erythrocytosis, such as *JAK2* mutation, pancytosis, splenomegaly, increased neutrophil alkaline phosphatase score, and increased serum vitamin B12 level. These findings indicated the possibility of an EPO-secreting tumor, but the patient's serum EPO level was within the normal reference range. Because imaging studies showed that the patient had a colon cancer without distant metastases, which could cause erythrocytosis, we managed the case with diagnostic treatment. If the erythrocytosis had sustained after the colectomy and revealed no secondary erythrocytosis, we would have had to consider primary erythrocytosis without *JAK2* mutation, secondary familial or congenital erythrocytosis, or idiopathic erythrocytosis. Bone marrow biopsy would have been needed to search other mutations causing these diseases [1]. However, her erythrocytosis subsided after the curative resection of the colon cancer, and the specimen tested positive for EPO-antibody. These findings showed this was indeed a rare case of EPO-secreting colon cancer.

The second clinical issue pertinent to this case is that it is important to consider not only the absolute serum EPO level but also the serum EPO level relative to simultaneously measured hemoglobin level. The patient's serum EPO level itself did not seem elevated, but an EPO 12.6 IU/L is actually relatively high considering her hemoglobin at 191 g/L, because serum EPO

level decreases when hemoglobin level increases. The relationship between serum EPO and hemoglobin levels has been examined in several studies, but only a few erythrocytosis cases were included [13–16]. Based on these limited data, serum EPO level at least 10 IU/L by enzyme immunoassay might be relatively high compared with high hemoglobin level, but the appropriate cut-off level requires further investigation. In short, when evaluating serum EPO level, we should always also consider the relative hemoglobin level. Serum EPO level just within the normal reference range is not sufficient to rule out an EPO-secreting tumor. In fact, paraneoplastic erythrocytosis of renal cell carcinoma, 12 of 14 cases, did not show high serum EPO level [17]. However, other tumor products besides ectopic EPO are also considered to cause paraneoplastic erythrocytosis. The products in question are the stimulator of renal EPO, the enhancer of EPO action, and the non-EPO stimulator of erythropoiesis [3]. Increased EPO level does not always mean that the tumor produces EPO. For instance, prostaglandins and testosterone produced by tumors may be important in stimulating EPO on responsive cells [18,19]. These products may shift the natural balance of EPO and hemoglobin. Immunohistochemical study helps to diagnosis EPO-secreting tumor when examining the cause of erythrocytosis with tumor [20]. Even if we have a tumor together with high serum EPO level, we cannot always be sure that it is actually an EPO-secreting tumor. The important thing is to confirm the local production of EPO. The focal presence of erythropoietin was shown in the colon adenocarcinoma cells in this case. In contrast, if the test is negative for EPO-antibody, the results may be a false negative or indicative of the effects of other mechanisms of paraneoplastic erythrocytosis.

We should include paraneoplastic erythrocytosis as a differential diagnosis in cases of high hemoglobin level unexplained by other diseases. Paraneoplastic syndrome can be the only symptom of cancer, and erythrocytosis is one of the rare paraneoplastic

syndromes. Effective diagnosis, such as colonoscopy, may substantially affect overall clinical outcomes. Paraneoplastic hematologic syndrome is rarely symptomatic but is typically seen in association with advanced disease [21]. The recognition can avoid overlooking a current cancer and save the patient excessive testing such as bone marrow examination.

Conclusions

Colon cancer can cause paraneoplastic erythrocytosis, and it is important to consider not only the absolute serum EPO level but also the serum EPO level relative to simultaneously measured hemoglobin levels. We should suspect paraneoplastic erythrocytosis including colorectal cancer in cases of high hemoglobin level unexplained by other disease, even if serum EPO level is within the normal reference range. It may be easily overlooked that asymptomatic cancer causes paraneoplastic erythrocytosis

with serum erythropoietin within the normal reference range, as in our case, because increased hemoglobin level masks an actually high serum EPO level. Immunohistochemical test is necessary to diagnose an EPO-secreting tumor, especially in this situation. The cut-off level of serum EPO with erythrocytosis requires further investigation.

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Conflict of interest

None declared.

References:

1. Lee G, Arcasoy MO: The clinical and laboratory evaluation of the patient with erythrocytosis. *Eur J Intern Med*, 2015; 26: 297–302
2. Doll DC, Weiss RB: Neoplasia and the erythron. *J Clin Oncol*, 1985; 3: 429–46
3. Hammond D, Winnick S: Paraneoplastic erythrocytosis and ectopic erythropoietins. *Ann NY Acad Sci*, 1974; 230: 219–27
4. Ban D, Sakamoto Y, Shimada K et al: Erythropoietin production caused by metastatic colon cancer. *Int J Colorectal Dis*, 2010; 25: 405
5. Lacombe C, Mayeux P: The molecular biology of erythropoietin. *Nephrol Dial Transplant*, 1999; 14: 22–28
6. Moritz KM, Lim GB, Wintour EM: Developmental regulation of erythropoietin and erythropoiesis. *Am J Physiol*, 1997; 273: 1829–44
7. Ebert BL, Bunn HF: Regulation of the erythropoietin gene. *Blood*, 1999; 94: 1864–77
8. Juul SE, Yachnis AT, Rojiani AM, Christensen RD: Immunohistochemical localization of erythropoietin and its receptor in the developing human brain. *Pediatr Dev Pathol*, 1999; 2: 148–58
9. Yasuda Y, Masuda S, Chikuma M et al: Estrogen-dependent production of erythropoietin in uterus and its implication in uterine angiogenesis. *J Biol Chem*, 1998; 273: 25381–87
10. Acs G, Acs P, Beckwith SM et al: Erythropoietin and erythropoietin receptor expression in human cancer. *Cancer Res*, 2001; 61: 3561–65
11. Kumar SM, Acs G, Fang D et al: Functional erythropoietin autocrine loop in melanoma. *Am J Pathol*, 2005; 166: 823–30
12. Gombos Z, Danihel L, Repiska V et al: Expression of erythropoietin and its receptor increases in colonic neoplastic progression: the role of hypoxia in tumorigenesis. *Indian J Pathol Microbiol*, 2011; 54: 273–78
13. Panjeta M, Tahirovic I, Karamelic J et al: The relation of erythropoietin towards hemoglobin and hematocrit in varying degrees of renal insufficiency. *Mater Sociomed*, 2015; 27: 144–48
14. Mercadal L, Metzger M, Casadevall N et al: Timing and determinants of erythropoietin deficiency in chronic kidney disease. *Clin J Am Soc Nephrol*, 2012; 7: 35–42
15. Artunc F, Risler T: Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease. *Nephrol Dial Transplant*, 2007; 22: 2900–8
16. Fehr T, Ammann P, Garzoni D et al: Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia. *Kidney Int*, 2004; 66: 1206–11
17. Ljungberg B, Rasmuson T, Grankvist K: Erythropoietin in renal cell carcinoma: Evaluation of its usefulness as a tumor marker. *Eur Urol*, 1992; 21: 160–63
18. Hagiwara M, McNamara DB, Chen IL, Fisher JW: Role of endogenous prostaglandin E2 in erythropoietin production and dome formation by human renal carcinoma cells in culture. *J Clin Invest*, 1984; 74: 1252–61
19. Bachman E, Travison TG, Basaria S et al: Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: Evidence for a new erythropoietin/hemoglobin set point. *J Gerontol A Biol Sci Med Sci*, 2014; 69: 725–35
20. Clark D, Kersting R, Rojiani AM: Erythropoietin immunolocalization in renal cell carcinoma. *Mod Pathol*, 1998; 11: 24–28
21. Pelosof LC, Gerber DE: Paraneoplastic syndromes: An approach to diagnosis and treatment. *Mayo Clin Proc*, 2010; 85: 838–54