VERSITA

Cent. Eur. J. Med. • 7(5) • 2012 • 650-654
DOI: 10.2478/s11536-012-0037-5

Central European Journal of **Medicine**

Severe aplastic anemia in a patient with rheumatoid arthritis after introduction of etanercept

Case Report

Yoshiro Horai*, Tomoya Miyamura, Karin Shimada, Motoko Ishida, Soichiro Takahama, Rumi Minami, Masahiro Yamamoto, Eiichi Suematsu

Department of Internal Medicine and Rheumatology, Clinical Research Center, National Hospital Organization Kyushu Medical Center, 1-8-1 Jigyohama Chuo-ku, Fukuoka 810-8563, Japan

Received 19 November 2011; Accepted 12 April 2012

Abstract: A 73-year-old female, diagnosed with rheumatoid arthritis (RA), complicated with severe levels of joint destruction, started etanercept (ETN) because of high persistent RA disease activity. Although her articular symptoms dramatically improved, she developed marked pancytopenia after the introduction of ETN. Bone marrow aspirate specimen revealed hypocellular marrow in three hematopoietic series without atypical findings, which was compatible with aplastic anemia (AA). This is a rare case of severe pancytopenia due to AA presumably induced by ETN.

Keywords: *Aplastic anemia • Rheumatoid arthritis • Etanercept*

1. Introduction

Tumor necrosis factor-α (TNF-α)-blocking agents have been recognized to be effective in the treatment of refractory rheumatoid arthritis (RA) in recent years. Etanercept (ETN) is a soluble TNF receptor fusion protein with immunoglobulin G1, which has been proven to be effective in combination with MTX or in monotherapy in the treatment of RA [1]. ETN, as well as TNF-α-blocking agents, shows excellent therapeutic effects against RA. However various adverse effects of ETN, especially reactivation of latent tuberculosis [2] and Pneumocystis jiroveci pneumonia [3] due to immunosuppression were widely known. Furthermore, several reports suggest that TNF-α-blocking agents may induce hematological disturbances. Although several possible mechanisms have been suggested, the exact mechanism of cytopenia induced by TNF-α-blocking agents is not still clarified. As a possible hypothesis, fluctuations of serum TNF-α levels might be associated with apoptosis as well as necrosis and lead to hematological disorders; Jurinsic et al. reported that cell death effects of TNF-α with TNF receptor -1(TNFR-1) and TNFR-2 in Raji cells (human Burkitt lymphoma) [4].

In this study, we describe a 73-year-old female with RA accompanied by severe pancytopenia due to aplastic anemia, after introduction of ETN.

2. Case report

A 73-year-old female was diagnosed with RA based on the presence of morning stiffness, tenderness and swelling of systemic joints including the bilateral finger joints in a local hospital in December 1999. Although treated with several oral disease-modifying anti-rheumatic drugs (DMARDs) including methotrexate (MTX), high RA disease activity persisted. Administration of infliximab (IFX) and methotrexate (8 mg/week) in October 2003 and her arthralgia dramatically improved. A 160 mg dose of IFX was given intravenously after 2, 6 and 14 weeks and every 8 weeks thereafter. However, administration of IFX was discontinued in May 2005 because of skin eruptions. Subcutaneous injection of ETN (25 mg twice weekly) was started in July 2005. There were no contraindications to the use of IFX and ETN in this patient; there were no signs of active or latent tuberculosis infection on chest X-ray, tuberculin skin test was negative, serum hepatitis B surface antigen and

* *E-mail: yoshirohorai0518@qmed.hosp.go.jp*

anti-hepatitis C antibodies were negative, electrocardiography revealed no abnormalities, neither malignant tumors nor demyelinating diseases were observed [5]. Slight anemia was found prior to the introduction of ETN, however, neither leukopenia nor thrombocytopenia were observed (white blood cell count, 6,600/μL; hemoglobin, 10.2 g/dl; platelet count, 184,000/μL; in June 2005). After the introduction of ETN, her articular symptoms improved. However, her platelet count was down to 73,000/μL and administration of ETN was stopped in February 2007. After that, MTX was also stopped due to the development of interstitial pneumonia which might be associated with MTX in March 2007. She was diagnosed with RA complicating idiopathic thrombocytopenic purpura, then Helicobacter pylori eradication therapy was done and 50 mg/day of prednisolone (PSL) was started as a therapy for interstitial pneumonia. The dose of PSL was then decreased gradually. The platelet count dropped to 32,000/μL in June 2007 and remained around 30,000/μL, although transiently increased to around 100,000/μL thereafter. After the termination of ETN, joint pain and swelling flared up again. Administration of tocilizumab (TCZ) (400 mg/body) was started in May 2008 and a 400 mg dose of TCZ was given intravenously every 4 weeks thereafter. After the sixth TCZ administration, leg ulcers which might be an adverse effect of TCZ, developed and TCZ was discontinued. The progress of multiple joint destructions was observed, and a left total shoulder arthroplasty was performed. Based on patient's strong will and enough informed consent, injections of ETN at 25 mg a week was resumed in mid March 2010 and her arthralgia was relieved. Four weeks after the resumption of ETN, her platelet count fell to 17,000/μL again. Administration of ETN was terminated again and she was referred to our department for further examination and treatment.

Physical examination at the referral to our department revealed synovitis of bilateral wrist and finger joints, compatible with persistent high disease activity of RA. Petechiae and subcutaneous bleeding were observed on her extremities. Subconjuctival hemorrhage and conjunctival pallor were observed. Cervical lymph nodes were not palpable. The results of laboratory tests were: white blood cell count, 4,100/μL (neutrophils 77.0%, lymphocytes 2.0%, monocytes 4.0%); hemoglobin, 7.6 g/dl; platelet count, 17,000/μL; lactate dehydrogenase, 342 IU/l; aspartate aminotransferase, 16 IU/L; alanine aminotransferase, 14 IU/L; alkaline phosphatase, 273 IU/L; γ-glutamyl transpeptidase, 12 IU/L, prothrombin time, 11.9 seconds; international normalized ratio, 0.90; activated partial thromboplastin time, 21.6 seconds; C-reactive protein (CRP), 0.48 mg/dl; erythrocyte sedimentation rate, 97 mm/hour; rheumatoid factor, 275 IU/mL; Matrix Metalloproteinase-3, 196.7 ng/mL; anti-double-stranded DNA antibody was negative (< 12 U/mL). Tests for protein and occult blood in urine were both negative. The microscopic examination of the urine sediment demonstrated neither red blood cells nor white blood cells. Anemia due to subcutaneous bleeding was difficult to control and she became transfusion-dependent. Computed tomography (CT) revealed interstitial infiltrate in the lower lung fields but negative for thoracic and abdominal lymphadenomegaly. The bone marrow aspirate was markedly hypocelluar but there were no morphologic features of dysplasia, which were compatible with AA. In addition, large granular lymphocytes (LGLs) were detected in bone marrow smears (Figure 1). LGLs were also found in peripheral blood smears (Figure 2).

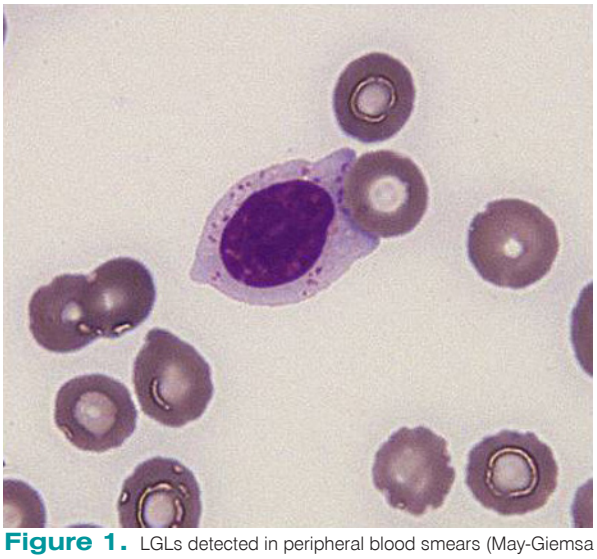

Figure 1. LGLs detected in peripheral blood smears (May-Giemsa stain, ×1,000).

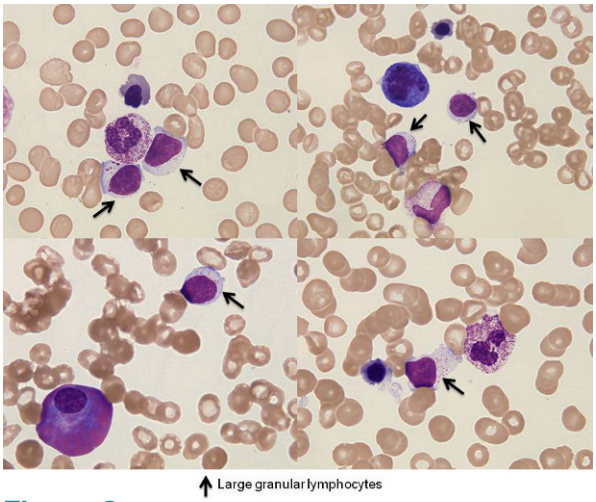

Figure 2. Bone marrow specimen; showing hypocellular bone marrow without dysplasia and large granular lymphocytes (LGLs) (May-Giemsa stain, ×1,000).

The presence of EBV genome in LGLs was not detected in situ hybridization study. LGL count gradually increased to 840/μL at the highest during the admission. Immunophenotyping of LGLs demonstrated T-cell phenotype and NK-cell phenotype almost equally. Southern blot analysis for TCR gene rearrangement did not reveal rearranged bands of the TCR-Cβ gene. Serological tests for EBV were compatible with past infection (anti-viral capsid antigen (VCA) -IgG, 1:320; anti-VCA-IgA, 1:10; anti-VCA-IgM, <1:10; anti-early antigen (EA) -IgG, 1:10; anti-EA-IgA, <1:10; anti-Epstein-Barr nuclear antigen-IgG antibody, 1:20). Quantification of plasma EBV- deoxyribo- nucleic acid (DNA) (real-time polymerase chain reaction (PCR)) revealed high EBV-DNA copy numbers (295 copy /106cells). Cytomegalovirus antigenemia tests were negative. We diagnosed the current patient with pancytopenia due to AA which might be associated with GLPD, and started oral 50 mg/day of cyclophosphamide (CPA). After the start of CPA, LGL count decreased to 100/μL. Although pancytopenia persisted, she remains in stable condition with transfusion of packed red blood cells per two to three weeks. The count of LGLs decreased after the start of CPA (Table 1).

3. Discussion

Although TNF-α-blocking agents, including ETN, have brought about a revolution in the treatment of RA and other inflammatory diseases, various adverse effects associated with ETN have been reported. Accoding to postmarketing surveillance of ETN, conducted by Japan College of Rheumatology, most common adverse effects are injection site reaction and rash. Other than these, severe adverse effects, such as pneumonia and malignant tumors were reported [6]. Furthermore, other events such as demyelinating diseases, autoimmune disorders and hematological disorders are often found [7]. Kaushik et al. reported a case of Ankylosing spondylitis complicating membranous glomerulonephritis after the introduction of ETN, and they suggested the possibility of immune dysregulation by ETN with subsequent MGN [8].

AA is divided into two groups, idiopathic or secondary. The causes of seconday AA include drug-induced, radiation and immunological factors. RA and anti-rheumatic drugs are considered to be risk factors for AA, respectively. Especially, anti-rheumatic drugs such as gold salts, D-penicillamine and non-steroidal anti-inflammatory drugs (NSAIDs) are thought as causes of drug-induced AA [9]. When patients with RA treated with anti-rheumatic drugs are suffering from AA, it can be difficult to distinguish between RA-related AA and drug-induced AA. Although there were other possible causes, such as RA itself and other drugs, we diagnosed the current patient with AA associated with ETN based on the fact that severe pancytopenia occurred in accordance with the introduction of ETN and pancytopenia reworsened when administration of ETN was resumed.

The mechanism of ETN-associated cytopenia is not fully understood. Pathare et al. suggested three possible mechanisms [10]. First, a cytokine-mediated mechanism; TNF-α regulates granulocyte macrophage colony-stimulating factor, therefore Blockade of TNF-α is thought to suppress stem-cell differentiation. Alternatively, drug-induced lupus-like syndrome is caused by TNF-α-blocking agents. Finally, TNF-α-blocking agents have direct toxic effects on the bone marrow. Jurisic et al. reported that a case of myelofiblosis with elevated serum TNF-α levels. Fibrosis is mediated by cytokine network including TNF-α [11]. Therefore, fluctuation of serum TNF-α concentration with TNF-α-blocking therapy might be involved in the pathogenesis of hematologic disturbances in patients with RA.

AA after introduction of ETN has rarely been reported. To the best of our knowledge, there have only one report described by Kuruvilla J. et al. [12]. While

Table 1. Laboratory data and duration of etanercept, tocilizumab and cyclosporine.

	ETN 25mg x 2/week				TCZ 400mg/4 weeks		ETN 25mg x 2/week					
	Jul-06	Feb-07	Jun-07	Oct-07	May-08	Dec-08	Mar-10	Apr-10	Sep-10		Oct-10	
WBC (/μL)	6,600	2,800	4,700	4,500	4,900	3,200	4,900	4,100	2,500	3,000	3,200	2,600
LGU (/μL)									550	840	740	100
Hb (g/dL)	10.2	8.5	6.6	9.5	9.4	8.5	7.6	7.8	8.1	7.9	7.4	8.6
Plt (/μL)	184,000	73,000	32,00	105,000	48,000	59,000	35,000	17,000	15,000	15,000	15,000	14,000
	CsA 50mg/day → → → →											

Abbreviations: CsA, cyclosporine; ETN, etanercept; Hb, hemoglobin; LGL, large granular lymphocyte; Plt, platelet count; TCZ, tocilizumab; WBC, white blood cell count.

white blood cell count, haemoglobin and platelet count had normalized after the termination of ETN in their case, no recovery of blood cell counts were observed even after the termination of ETN and the start of immnosuppressive therapy in the presented our case. The difference between the previously reported case by Kuruvilla J et al. and the presented our case may be due to total dosage of ETN; about 5 g of ETN was given until pancytopenia developed in the presented our case, while 1 g in the case described by Kuruvilla J et al. This fact suggested that accumulated bone marrow toxicity played an important role in the ETN-associated AA, and it seemed important to discontinue ETN quickly if cytopenia developed during ETN therapy.

One possible cause of pancytopenia in this case is reactivation of EBV, because high EBV-DNA copy numbers were detected. It is known that EBV infection can become a cause of AA. According to the analysis reported by Balanrraud N et al., eleven of forty-eight patients with RA treated with ETN revealed increase of EBV viral load, despite they concluded it was not statistically significant change [13]. There are two reports describing EBV-activation-associated adverse events of TNF-α-blocking agents. One is EBV-associated Hodgkin-like disease developed after the start of infliximab (IFX), chimeric monoclonal antibody against TNF-α [14], and the other is EBV-reactivation-associated disseminated encephalomyelitis during IFX [15].

An interesting feature of this case is that relatively-high LGLs were found in the peripheral blood and bone marrow smears, which suggested the possibility of granular lymphocyte proliferative disorder (GLPD). This case did not meet completely the conventional criteria for GLPD [16], because the count of LGL was less than 2,000/μL, a monoclonal proliferation of LGL could not be proven with southern blot analysis for TCR gene rearrangement and EBV genome in LGLs was not detected in situ hybridization study. However, it is suggested that GLPD may develop even with LGLs less than 2,000/μL, and may appear polyclonally [16]. Felty' s syndrome (FS) is a condition characterized by RA, neutropenia and splenomegaly. This case showed RA and pancytopenia, but the lack of splenomegaly denied the diagnosis of Felty's syndrome. However, it is suggested that FS and GLPD are different variants of syndrome comprising RA, neutropenia, splenomegaly, LGL proliferation and positiveness of human leukocyte antigen-DR4 [17]. In addition, it is reported that chronic or reactivated EBV infection is associated with GLPD [18]. Therefore pancytopenia may be compounded by GLPD-like mechanism. Further studies are warranted to disclose a relation between GLPD and pancytopenia in patients with RA.

Triggering receptor expressed on myeloid cell-1 (TREM-1) is a cell surface receptor expressed on monocytes and neutrophils, and blockade of TREM-1 with a fusion protein containing murine TREM-1 extracellular domain with immunoglobulin G1 reduces serum TNF-α levels. Furthermore, blockade of TREM-1 did not affect immune responses against bacterial infection [19]. Therefore TREM-1-blocking therapy might be a safe and effective therapeutic option for RA in the future.

In this study, we report a case of AA in the patient with RA after the start of ETN. We should be aware that ETN may induce severe pancytopenia due to AA. Further studies will be needed to clarify the exact mechanism of ETN-associated cytopenia.

Conflict of interest

The authors declare they have no conflict of interest statement.

References

[1] van der Heijde D, Klareskog L, et al. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. Arthritis Rheum, 2006, 54: 1063-1074

[2] Katikireddi VS, Whittle SL, Hill CL. Tumour necrosis factor inhibitors and risk of serious infection in rheumatoid arthritis. Int J Rheum Dis, 2010, 13: 12-26

[3] Komano Y, Harigai M, Koike R, et al. Pneumocystis jiroveci pneumonia in patients with rheumatoid arthritis treated with infliximab: a retrospective review and case-control study of 21 patients. Arthritis Rheum, 2009, 61: 305-312

[4] Jurisic V, Bogdanovic G, Kojic V, Jakimov D, Srdic T. Effect of TNF-alpha on Raji cells at different cellular levels estimated by various methods. Ann Hematol. 2006, 85, 86-94

[5] Miyasaka N, Takeuchi T, Eguchi K. Guidelines for the proper use of etanercept in Japan. Mod Rheumatol, 2006, 16: 63-67

[6] Koike T, Harigai M, Inokuma S, et al. Postmarketing surveillance of the safety and effectiveness of etanercept in Japan. J Rheumatol, 2009, 36: 898-906
[7] Takeuchi T, Amano K. Etanercept (in Japanese). Nippon Rinsho, 2002, 60: 2390-2396
[8] Kaushik P, Rahmani M, Ellison W. Membranous glomerulonephritis with the use of etanercept in ankylosing spondylitis. Ann Pharmacother, 2011, 45:e62
[9] Baumelou E, Guiguet M, Mary JY. Epidemiology of aplastic anemia in France: a case-control study. I. Medical history and medication use. The French Cooperative Group for Epidemiological Study of Aplastic Anemia. Blood, 1993, 81:1471-1478
[10] Pathare SK, Heycock C, Hamilton J. TNFalpha blocker-induced thrombocytopenia. Rheumatology (Oxford), 2006, 45:1313-1314
[11] Jurisic V, Terzic T, Pavlovic S, Colovic N, Colovic M. Elevated TNF-alpha and LDH without parathormone disturbance is associated with diffuse osteolytic lesions in leukemic transformation of myelofibrosis. Pathol Res Pract. 2008, 204, 129-132
[12] Kuruvilla J, Leitch HA, Vickars LM, Galbraith PF, Li CH, Al-Saab S, et al. Aplastic anemia following administration of a tumor necrosis factor-alpha inhibitor. Eur J Haematol, 2003, 71: 396-398
[13] Balandraud N, Guis S, Meynard JB, Auger I, Roudier J, Roudier C. Long-term treatment with methotrexate or tumor necrosis factor alpha inhibitors does not increase epstein-barr virus load in patients with rheumatoid arthritis. Arthritis Rheum, 2007, 57: 762-767
[14] Komatsuda A, Wakui H, Nimura T, Sawada K. Reversible infliximab-related lymphoproliferative disorder associated with Epstein-Barr virus in a patient with rheumatoid arthritis. Mod Rheumatol, 2008, 18, 315-318
[15] Ueda M, Tateishi T, Shigeto H, Yamasaki R, Ohyagi Y, Kira J. A case of acute disseminated encephalomyelitis associated with Epstein-Barr virus reactivation during infliximab therapy (in Japanese). Rinsho Shinkeigaku, 2010, 50: 461-466
[16] Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran TP Jr. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. Blood, 1997, 89:256-260
[17] Bowman SJ, Sivakumaran M, Snowden N, et al. The large granular lymphocyte syndrome with rheumatoid arthritis. Immunogenetic evidence for a broader definition of Felty's syndrome. Arthritis Rheum. 1994, 37, 1326-1330
[18] Hart DN, Baker BW, Inglis MJ, et al. Epstein-Barr viral DNA in acute large granular lymphocyte (natural killer) leukemic cells. Blood, 1992, 79: 2116-2123
[19] Kim TH, Choi SJ, Lee YH, Song GG, Ji JD. Soluble triggering receptor expressed on myeloid cells-1 as a new therapeutic molecule in rheumatoid arthritis. Med Hypotheses. 2012, 78, 270-272