Successful azacitidine therapy for myelodysplastic syndrome associated with VEXAS syndrome

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Running head: Azacitidine for MDS with VEXAS syndrome

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Abstract

VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is caused by *UBA1* somatic mutations and is characterized by late-onset systemic autoimmune inflammation and blood abnormalities such as cytopenia, vacuolation of myeloid/erythroblastic cells, and myelodysplastic syndrome (MDS). It is often resistant to immunosuppressive therapy, and no treatment strategy has been established. A 65-year-old man presented with palpable erythema, fever, macrocytic anemia, and arthralgia. He was subsequently diagnosed with MDS complicated by Sweet's disease. Treatment with azacitidine was initiated due to suspected skin invasion by MDS cells and resistance of the skin rash to steroid therapy. Next-generation sequencing of bone marrow samples prior to treatment initiation revealed the presence of *UBA1* p.M41L (VAF 0.38) and *DNMT3A* p.L605fs mutations (VAF 0.184). Based on the findings of systemic inflammation, a diagnosis of VEXAS syndrome was made. The fever and skin rash improved with azacitidine therapy. In conclusion, somatic mutations in *UBA1* should be explored in patients with MDS exhibiting systemic autoimmune inflammation. Furthermore, azacitidine may be a good treatment option for systemic autoinflammation in MDS associated with VEXAS syndrome.

Keywords: VEXAS syndrome; myelodysplastic syndrome; azacitidine*; UBA1* mutation*; DNMT3A* mutation

Statements and Declarations

Introduction

The VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome, a new disease concept proposed by Beck et al. in 2020, is a treatment-resistant autoinflammatory disease that develops in late adulthood owing to somatic mutations in *UBA1*, causing fever, neutrophilic dermatitis/pneumonia, arthritis, and hematologic abnormalities, such as cytopenia, vacuolation of myeloid/erythroblastic cells, and myelodysplastic syndrome (MDS) [1]. VEXAS syndrome is caused by *UBA1* mutations in hematopoietic stem cells, which induce systemic inflammation. This new disease concept was discovered by analyzing 25 patients with *UBA1* p.Met41 mutations identified by exome sequencing. The disease was named VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome as an acronym for the disease characteristics. All patients were male and had old-onset systemic inflammatory and hematological abnormalities. The effects of steroids are often limited, and no disease-specific treatment has been proposed [1, 2]. Herein, we present a patient with MDS associated with VEXAS syndrome, successfully treated with azacitidine.

Case

A 65-year-old man exhibited palpable erythema. Upon suspicion of drug eruption, oral hypoglycemic agents were changed; however, no improvement was observed, and a temperature of 38°C and arthralgia appeared. No weight loss, chondritis, ocular, respiratory, or gastrointestinal symptoms were observed. Suspecting adult Still's disease, the patient was started on a combination tablet of betamethasone/d-chlorpheniramine maleate (5 mg prednisolone equivalent dose/day), topical steroid, and tranilast, demonstrating an improvement in symptoms; however, palpable erythema and fever

flared up following dosage reduction (Figure1). He was diagnosed as histiocytoid Sweet syndrome by skin biopsy, and prednisolone 30 mg was initiated. In addition, blood tests revealed a hemoglobin level of 10.7 g/dL, mean corpuscular volume of 110.8 fL, white blood cell count of 4670/μL, and platelet count of 199000/μL, indicating macrocytic anemia (Table1). Bone marrow aspiration showed no blasts, but there were three lines of dysplasia: micromegakaryocytes, Pseudo-Pelger-Huet anomaly, and megaloblast-like changes. Myelocytes and Monocytes with vacuoles were also observed (Figure2). The karyotype of the patient was 46, XY. The patient was diagnosed with MDS (Revised International Prognostic Scoring System 1 point, low risk) and was followed up without treatment.

However, on reducing the dose to 20 mg prednisolone, the skin rash and fever flared, and potassium iodide 900 mg and colchicine 1 mg were added, but with little effect. A skin biopsy was performed again, and an infiltrate of myeloperoxidase (MPO), CD68 and CD163 positive immature myeloid cells was detected, which was considered a skin infiltrate of MDS (Figure3). There were no findings of venous thrombosis. In addition, the targeted sequence of the bone marrow aspirate presented *DNMT3A* p.L605fs (VAF 0.184) and *UBA1* p.M41L (VAF 0.380) mutations. Based on the systemic inflammation, a diagnosis of VEXAS syndrome was made. The patient received azacitidine (75mg/m^2 subcutaneous injection, once daily for 7 days in a 4-week schedule), and the fever and skin rash showed improvement. Blood test data at the start of azacitidine treatment are shown in Table 2. Six courses of azacitidine were continued, and the prednisolone dose was reduced to 10 mg without the reappearance of any symptoms. In addition, blood counts did not show progressive cytopenia (Figure4) or the appearance of blasts. Bone marrow aspiration after six courses of azacitidine treatment showed three lineage

dysplasia without significant change from pre-treatment, but no increase in blasts. Based on the above, the patient was judged to be SD (stable disease) according to the 2006 IWG response criteria.

Discussion

Herein, we present a case report of MDS with macrocytic anemia, fever, palpable erythema, and arthralgia, finally diagnosed as VEXAS syndrome by next-generation sequencing. Symptoms such as palpable erythema and fever were resistant to prednisolone therapy but were successfully treated with azacitidine.

In the first report of VEXAS syndrome, 6 of 25 patients were diagnosed with MDS [1]. In another study examining 15 patients with VEXAS syndrome, 5 were diagnosed with MDS [3]. These findings suggest that the combination of VEXAS syndrome and MDS is relatively common, although exact statistics remain unavailable. VEXAS syndrome is often resistant to immunosuppressive therapy [4], and it is important to identify VEXAS syndrome for considering potential secondary therapy.

To date, no disease-specific treatment for VEXAS has been proposed. The present case report suggests that azacitidine for treating MDS associated with VEXAS syndrome could improve systemic inflammation. Previously, 29 patients with MDS/chronic myelomonocytic leukemia with systemic autoimmune and inflammatory diseases were treated with azacitidine to improve inflammatory findings; however, there was no significant difference in efficacy between patients with and without *UBA1* mutations [5]. There are also reports of success with Azacitidine in myelodysplastic syndrome and myofasciitis due to VEXAS syndrome [6]. These findings, along with our experience,

suggest that azacitidine may be a reasonable treatment for VEXAS syndrome. In addition to *UBA1* mutation, the present patients presented with *DNMT3A* mutation. *DNMT3A* and *TET2* mutations are frequently associated with MDS due to VEXAS syndrome. Several cases of azacitidine response in VEXAS syndrome patients with these mutations have been reported [1, 7, 8]. *DNMT3A* mutations are a known cause of age-related clonal hematopoiesis and one of the causes of hematologic malignancies [9, 10]. Moreover, it is known that certain gene mutations, including *DNMT3A* mutations, upregulate the expression of inflammatory cytokines such as interleukin (IL)-6, TNF-α, and IL-13. *DNMT3A* mutations, found in some patients with VEXAS, may contribute to inflammation [7, 11-13], which may in turn expand the clones harbored with *DNMT3A* mutations.

Somatic mutation in *UBA1* includes p.Met41Thr, p.Met41Val, p.Met41Leu and splice mutations. *UBA1* p.Met41Leu mutation is reported to be more frequent in patients with mild to moderate VEXAS syndrome and has a better 5-year survival rate compared to patients with other *UBA1* mutations [14]. On the other hands, MDS with *DNMT3A* mutation is considered to have a poor prognosis [15]. The prognosis of VEXAS syndrome with *UBA1* p.Met41Leu mutation and *DNMT3A* mutation is unknown, but the prognostic determinant in this case is expected to be MDS rather than VEXAS syndrome. Allogenic hematopoietic stem cell transplantation can be considered because there are a few reports showing the efficacy of transplantation for systemic inflammation in VEXAS syndrome [16], and because it can aim for cure of MDS.

In this case, histiocytoid sweet syndrome was observed as a cutaneous manifestation of VEXAS syndrome, and it has been reported that in the sweet syndrome-like rash seen in MDS associated with VEXAS syndrome, most infiltrating cells are CD68-positive,

myeloperoxidase-positive histiocyte-like bone marrow progenitor cells[17]. Zakine et al. noted that in addition to mature neutrophils, the infiltrate contains a mixture of lymphoid and immature myeloid cells, and paired sequencing analysis revealed that the skin infiltrate in VEXAS syndrome is derived from the same pathological clone as the UBA1 mutant myeloid cells in the bone marrow[18]. This was confirmed by azacitidine-treated skin infiltrates. This may be one reason why the skin rash improved with azacitidine administration.

Currently, no treatment strategy has been established for VEXAS syndrome, which remains intractable. In the present case report, the skin rash and fever improved after administering azacitidine. Azacitidine administration may be a suitable option for treating VEXAS syndrome.

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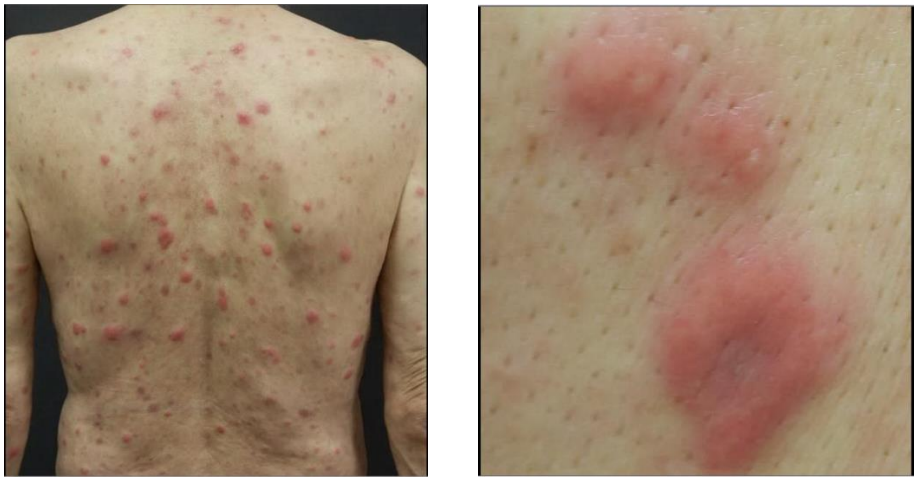
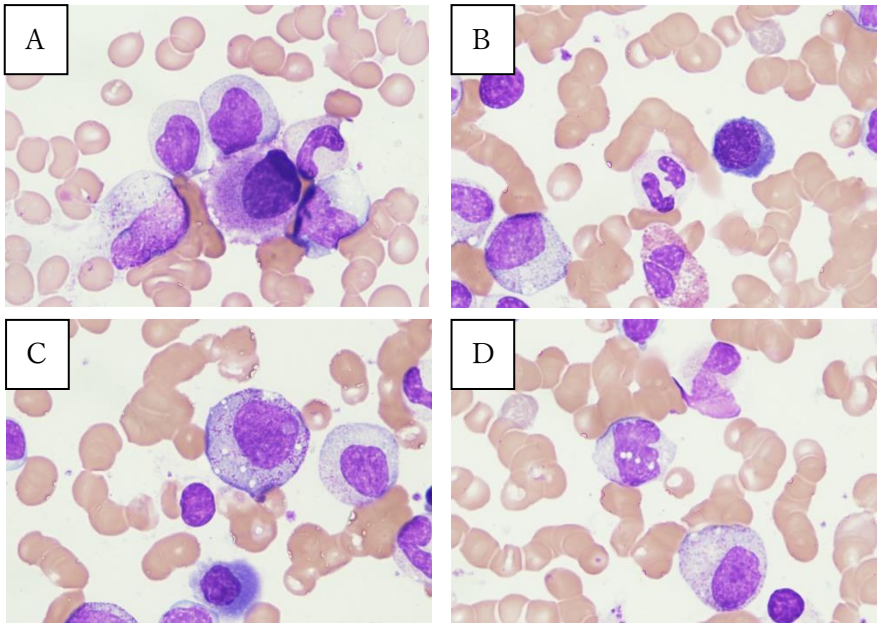
Figure legends

Figure 1 Skin rash when skin rash flares up after discontinuation of prednisolone



Palpable erythema with some blistering appeared on the trunk of the extremities.

Figure 2



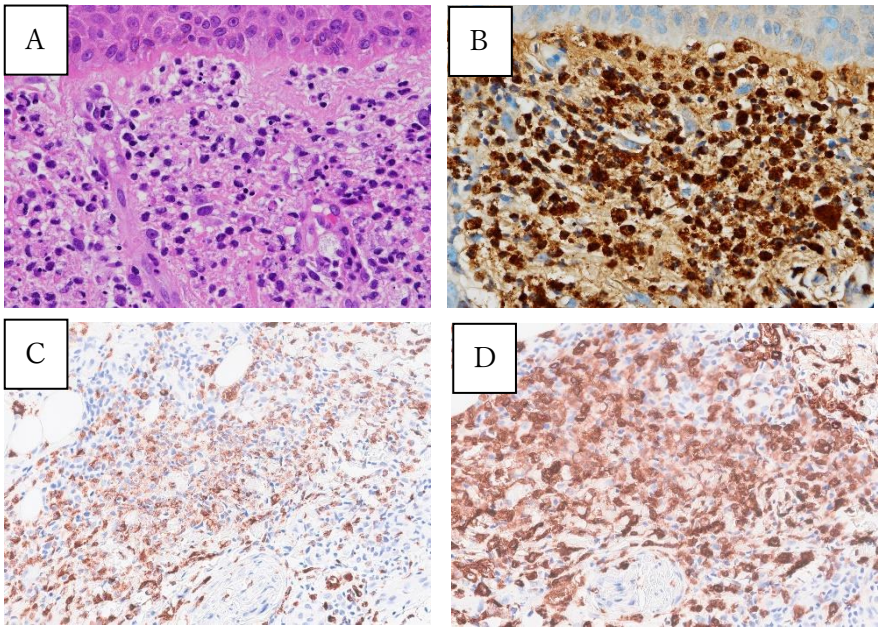
Bone marrow aspiration shows three linage dysplasia and vacuolation of myeloid/monocytic cells.

A: Micromegakaryocyte

B: Pseudo-Pelger-Huet anomaly and megaloblast-like change

C, D: Vacuoles in a myelocyte and monocyte

Figure 3 Skin biopsy when skin rash flares up after discontinuation of prednisolone

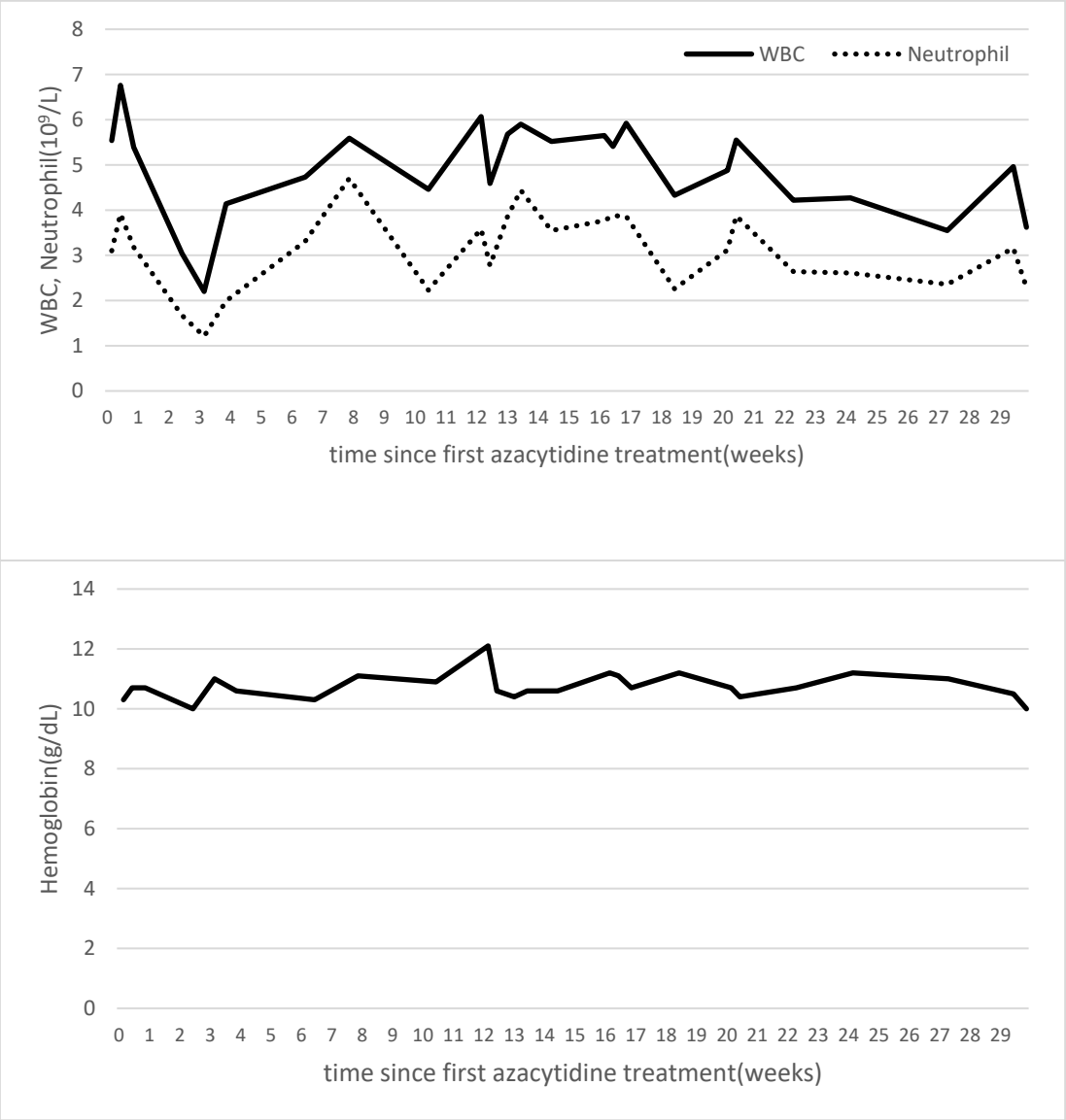


Histiocytoid mononuclear cells infiltrate the superficial dermis and nuclear debris is seen (A: H&E, x400).

The histiocytoid cells are diffusely positive for MPO (B: MPO, x400), CD68 (C: CD68, x400), CD163 (D: CD163, x400).

Immunostaining of the first skin biopsy had similar results.

Figure 4 Trends in blood counts after azacitidine initiation



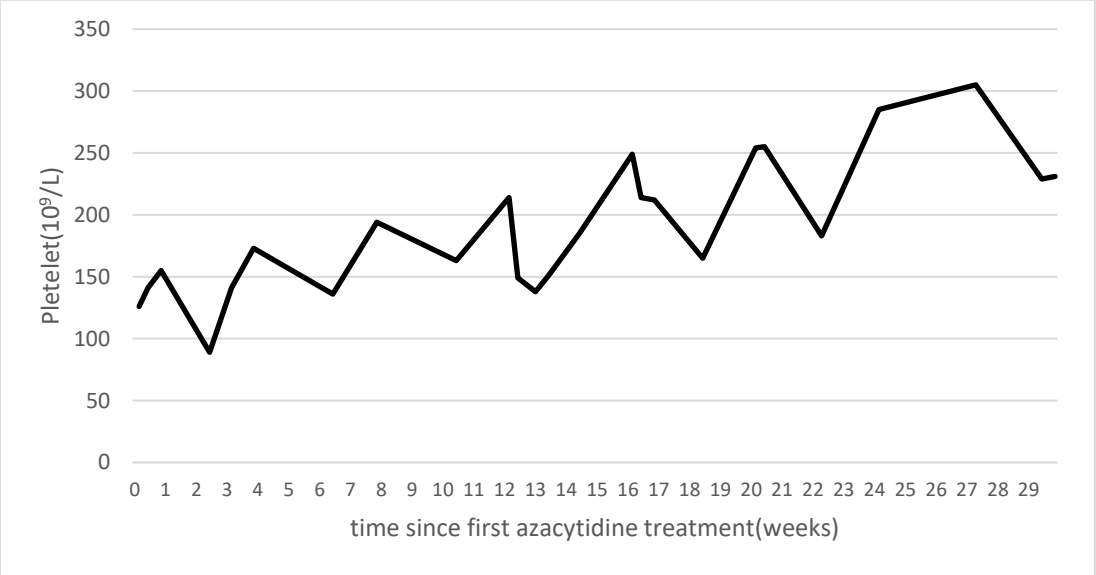

350
300
250
200
150
100
50
0
Pletelet(10⁹/L)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
time since first azacytidine treatment(weeks)

Table 1 Blood test data at the time of diagnosis of histiocytoid Sweet syndrome

WBC	4.67	10^9/L		AST	28	U/L		IgG	2620	mg/dL
RBC	2.95	10^12/L		ALT	16	U/L		IgA	892	mg/dL
Hb	10.7	g/dL		LDH	285	U/L		IgM	75	mg/dL
Ht	32.7	%		ALP	72	U/L		C3	161.9	mg/dL
MCV	110.8	fL		TP	7.9	g/dL		C4	20.5	mg/dL
MCH	36.3	pg		Albmin	2.9	g/dL		CH50	>60	CH50/mL
MCHC	32.7	%		T-Bil	0.7	mg/dL		haptgrobin	255	mg/dL
PLT	199	10^9/L		Cre	0.55	mg/dL		ferritin	681.2	ng/dL
Reticulocyte	19.8	‰		UA	3.8	mg/dL		β2-microgrobrin	3.86	mg/L
	0.06	10^12/L		UN	8	mg/dL		procalcitonin	0.09	ng/mL
FRC	0.2	%		T-Cho	128	mg/dL		sIL2-R	682	U/mL
				Na	141	mmol/L				
Neutrophil	64.6	%		K	3.4	mmol/L		protein fraction		
	3.01	10^9/L		Cl	105	mmol/L		Alb	38.2	%
Lymphocyte	27	%		Fe	70	μg/dL		α1	3.6	%
	1.26	10^9/L		UIBC	148	μg/dL		α2	10.3	%
Monocyte	6.9	%		CRP	7.1	mg/dL		β1	5.7	%
Eosinophil	1.5	%						β2	9.4	%
Basophil	0.2	%		Vitamin B12	276	pg/mL		γ	32.8	%
				folic acid	3	ng/mL		A/G	0.6	
PT-INR	1.07									
APTT	39.5	sec		antinuclear antibody	<40	times		free light chein κ	107.5	mg/L
fibrinogen	474	mg/dL						free light chein λ	130.3	mg/L
D-dimer	2.5	μg/dL						κ / λ ratio	0.83	

Table 2 Blood test data at the start of azacitidine treatment

WBC	5.54	10^9/L		AST	28	U/L		PT-INR	1.44	
RBC	2.65	10^12/L		ALT	31	U/L		APTT	39.4	sec
Hb	9.7	g/dL		LDH	279	U/L		fibrinogen	555	mg/dL
Ht	29.2	%		ALP	85	U/L				
MCV	110.2	fL		TP	6.8	g/dL				
MCH	36.6	pg		Albmin	2.6	g/dL				
MCHC	33.2	%		T-Bil	0.6	mg/dL				
PLT	107	10^9/L		Cre	0.61	mg/dL				
Reticulocyte	21.8	‰		UA	3.9	mg/dL				
	0.06	10^12/L		UN	23	mg/dL				
FRC	0	%		T-Cho	157	mg/dL				
				Na	133	mmol/L				
Neutrophil	90	%		K	3.8	mmol/L				
	4.99	10^9/L		Cl	96	mmol/L				
Lymphocyte	6	%		CRP	11.6	mg/dL				
	0.33	10^9/L								
Monocyte	4	%								
Eosinophil	0	%								
Basophil	0	%								