CASE REPORT

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A novel mutation of *WAS* gene in a boy with *Mycobacterium bovis* infection in spleen

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Abstract

Wiskott-Aldrich syndrome (WAS) is a primary immunodeficiency disorder caused by mutations of the gene encoding WAS protein (WASp). A scoring system has been used to grade severity of the disease. However, the phenotype of the disease may progress over time, especially in children younger than 2 years of age.

Here, we report a male child who presented with X-linked thrombocytopenia (XLT). Mutation analysis revealed a novel hemizygous 13-bp deletion (c.181_193delGCTGAGCACTGGA) on exon 2 of the *WAS* gene. This frameshift mutation resulted in a premature terminating codon at position 71 (p.A61fsX10). Molecular analysis of maternal DNA revealed a heterozygosity of the same mutation. The disease progressed to classic WAS within 8 months. Later, gastric varices as a consequence of *Mycobacterium bovis* infection in the spleen was detected. The rapid worsening of the disease may be due to the severe genotype of this patient.

Keywords: Wiskott-Aldrich syndrome, normal-sized platelets, novel mutation, *Mycobacterium bovis*, gastric varices

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Introduction

Wiskott-Aldrich syndrome (WAS; OMIM 301000) is an X-linked recessive primary immunodeficiency disorder that is caused by mutations of the gene encoding WAS protein (WASp). Variations in clinical phenotype and overall survival are determined by the effect of the mutation on WASP expression, with genotype-phenotype correlation having been demonstrated.[1,2] Classic WAS is characterized by thrombocytopenia with small-sized platelets, eczema, recurrent infection, high incidence of autoimmunity, and increased risk of malignancies. The milder form, X-linked thrombocytopenia (XLT), is usually limited to thrombocytopenia with absent or minor infections and eczema.[3] A scoring system has been used to grade severity of clinical manifestations in these patients.[4,5] However, the phenotype of the disease may progress over time, especially in children younger than 2 years of age. Cases in patients aged less than 2 years require close observation to early detect changes in condition, so that appropriate changes to the management plan can be implemented.

Most patients with WAS/XLT have low mean platelet volume (MPV). However, patients with WAS/XLT with normal-sized platelets have been reported.[6,7]

Infection from Bacillus Calmette-Guerin (BCG) vaccine was previously reported in patients with severe combined immunodeficiency (SCID) disease, chronic granulomatous disease (CGD), and Mendelian susceptibility to mycobacterial diseases (MSMD), but rarely reported in patients with WAS.[8] The pathogenesis may be from restricted T-cell repertoires, migration defects, and impaired phagocytosis or oxidative burst.[8]

In this case report, we describe a child with classic WAS who initially presented with XLT. The disease progressed to classic WAS with autoimmune diseases within 8 months. *Mycobacterium bovis* infection caused fibrotic compression of the splenic vessels and he developed splenomegaly and gastric varices over time. The rapid worsening of the disease may have been due to the severe genotype of this patient.

Report of case

A Thai boy presented with bloody stool since 25 days of age. He also had generalized petechiae without hepatosplenomegaly and his platelet count was 18,000/mm^3. The provisional diagnosis was neonatal alloimmune thrombocytopenia. Maternal washed platelets were given. From 2 to 12 months of age, he developed intermittent petechiae and

persistent thrombocytopenia. Complete blood count showed Hb 12.4 g/dl, Hct 35.5%, WBC 13,700/mm^3, neutrophils 20%, lymphocytes 39%, monocytes 17%, eosinophils 19%, and platelets 56,000/mm^3 (MPV 7.1 fL). Bone marrow aspiration found increased number of megakaryocytes. Accordingly, a diagnosis of idiopathic thrombocytopenic purpura was made and he was treated with oral prednisolone.

Due to the persistent thrombocytopenia with chronic eczema (WAS score: 2), WAS was suspected when the patient was 15 months of age. Immunological study showed IgG 1,040 mg/dl, IgA 144 mg/dl, IgM 17.7 mg/dl, IgE 2,190 mg/dl, CD4 1,343/mm^3 (39.32%), CD8 175/mm^3 (5.12%), CD3 1,575/mm^3 (46.12%), CD19 261/mm^3 (7.63%), and NK cells 1,507/mm^3 (44.12%). Lymphocyte transformation assay to phytohemagglutinin was normal.

After informed consent was obtained, molecular analysis of the *WAS* gene was performed using DNA from the patient and his mother. Briefly, genomic DNA was extracted from peripheral blood lymphocytes. Twelve coding exons and exon-intron junctions of the *WAS* gene were amplified by PCR, subsequently subjected to direct DNA sequencing in both forward and reverse directions. Nucleotide and amino acid numbering of *WAS* were based on NM_000377.2 and NP_00 0368.1, respectively.

Mutation analysis of the *WAS* gene revealed a novel hemizygous 13-bp deletion (c.181_193delGCTGAGCACTG-GA) in exon 2 (**Figure 1**). This frameshift mutation resulted in a premature termination codon at position 71 (p.A61fsX10). Heterozygosity of the same mutation was identified in maternal DNA (**Figure 1**), indicating that the mutation was inherited from his carrier mother.

IVIG was then administered every 4 weeks. At 23 months of age, our patient developed anemia, splenomegaly, and numerous purple-colored patches on his hands, legs, and feet (**Figure2A**). Our investigation revealed the following: Hematocrit 24%; peripheral blood smear showed spherocyte and polychromasia/reticulocyte 4% and direct Coombs test was positive. The follow-up CD marker showed decreased CD4 to 267/mm^3 (33.78%), CD8 to 53/mm^3 (6.76%), and CD3 to 358/mm^3 (42.25%). Our patient then later developed frequent hematemesis. Critical gastric varices were identified via upper GI endoscopy. Doppler ultrasonography showed splenomegaly with a small number of hypoechoic nodules (size~1.3 cm with internal calcification (**Figure 2B**), no portal or splenic vein thrombosis. Abdominal CT scan found multiple small ill-defined hypodensity lesions in spleen, which raised suspicion of granulomatous infection (**Figure 2C**). Therefore, a percutaneous needle biopsy of the splenic nodule was performed under ultrasonographic guide. The culture of the biopsy specimen revealed *Mycobacterium bovis*. Antituberculosis medications were given to the patient, but he soon developed transaminitis and multiple episodes of massive hematemesis. An explore laparotomy was inevitable. The operative findings consisted of splenomegaly with multiple small calcified nodules extending to the splenic hilum and the splenic vessels sparing the pancreas. There were engorged upper pole vessels extending to the gastric fundus and cardia. A partial splenectomy, splenic hilum dissection and gastric devascularization was performed. Episodes of hematemesis discontinued after surgery. We restarted the antituberculosis medications and planned to follow up abdominal CT scan at the 6th month after surgery. Due to the progression from XLT

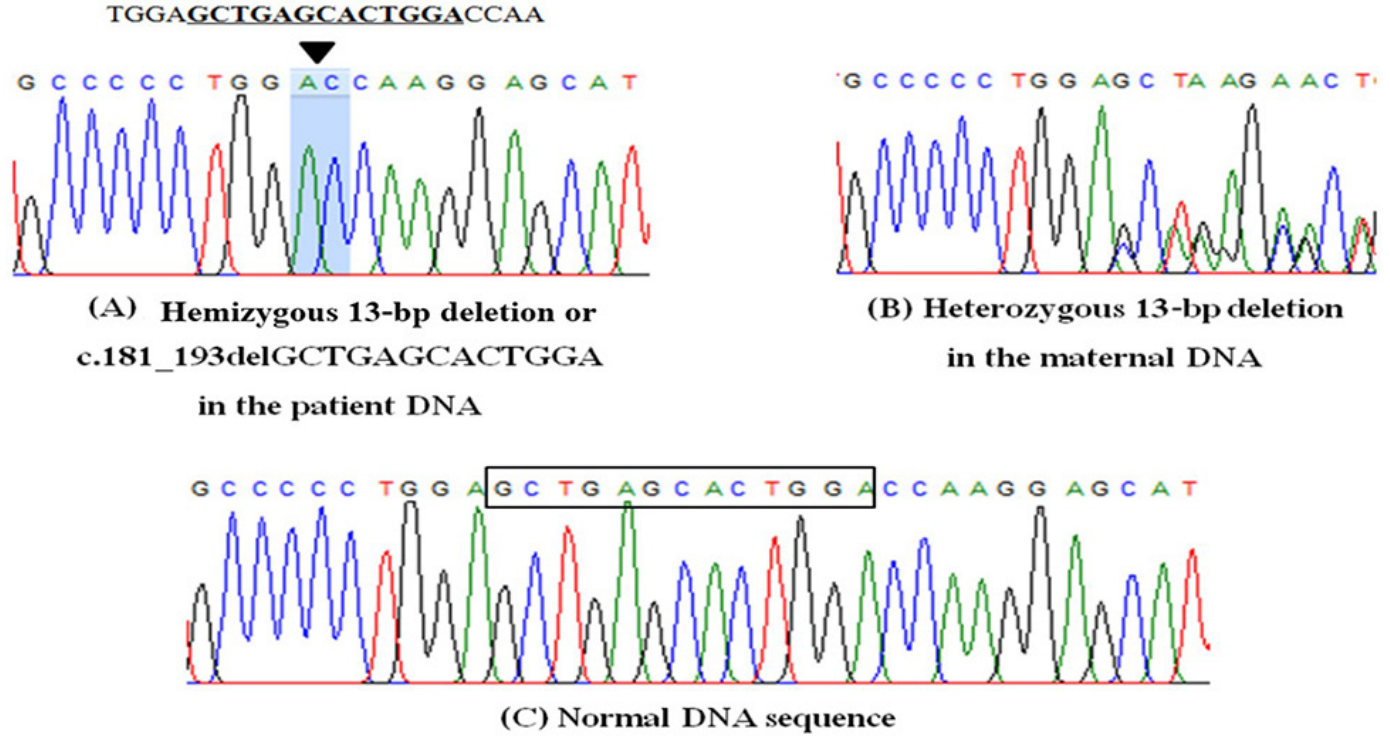

Figure 1. Direct DNA sequence analysis of exon 2 of *WAS* gene. (A) Direct DNA sequencing revealed a novel hemizygous 13-bp deletion (c.181_193delGCTGAGCACTGGA) in a patient with Wiskott-Aldrich syndrome; (B) A heterozygous 13-bp deletion in the proband's mother; (C) Normal DNA sequence

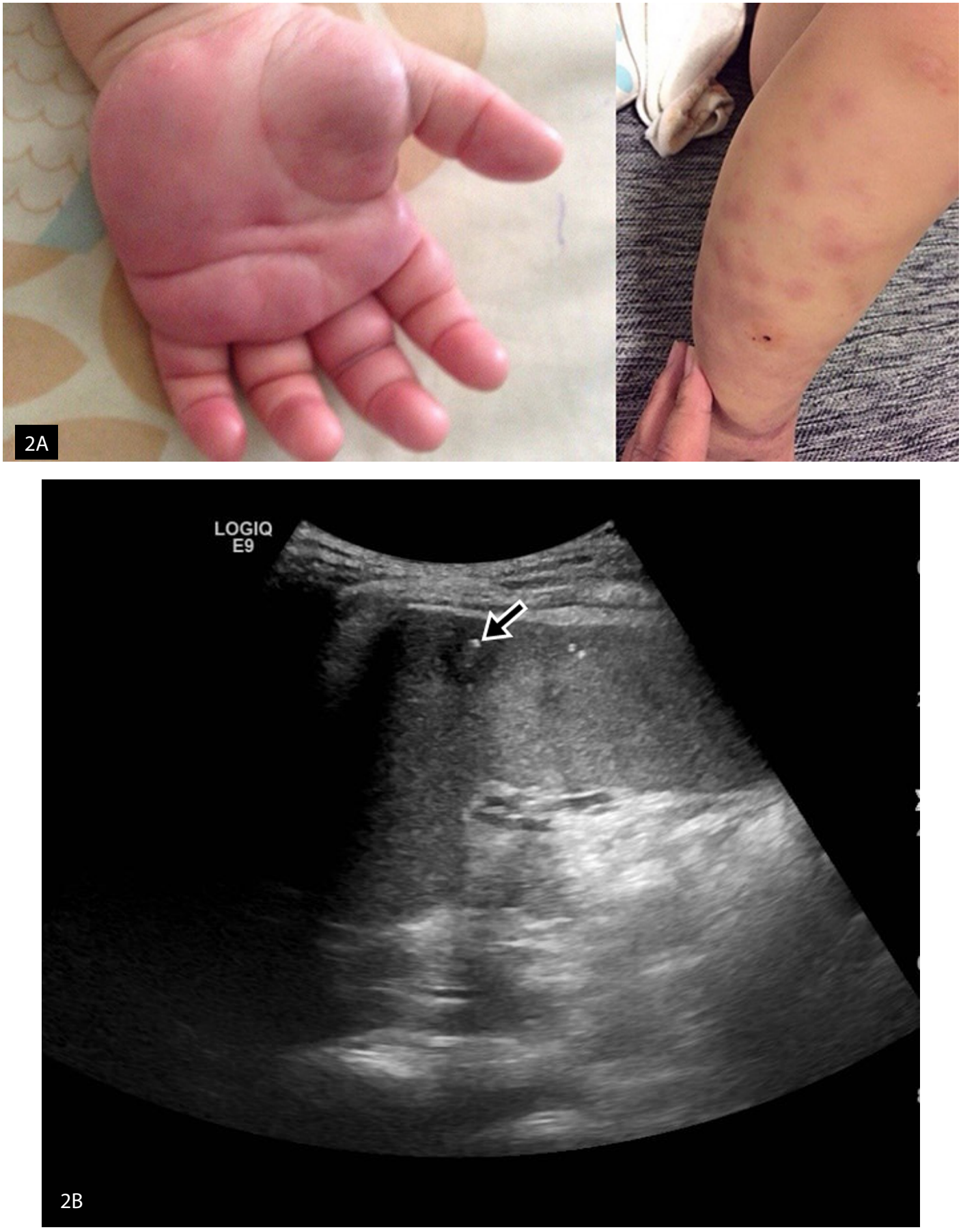

Figure 2. (A) Purple-colored patches on hands and legs; (B) Ultrasound showing hypoechoic nodules with internal calcification (arrow) in spleen

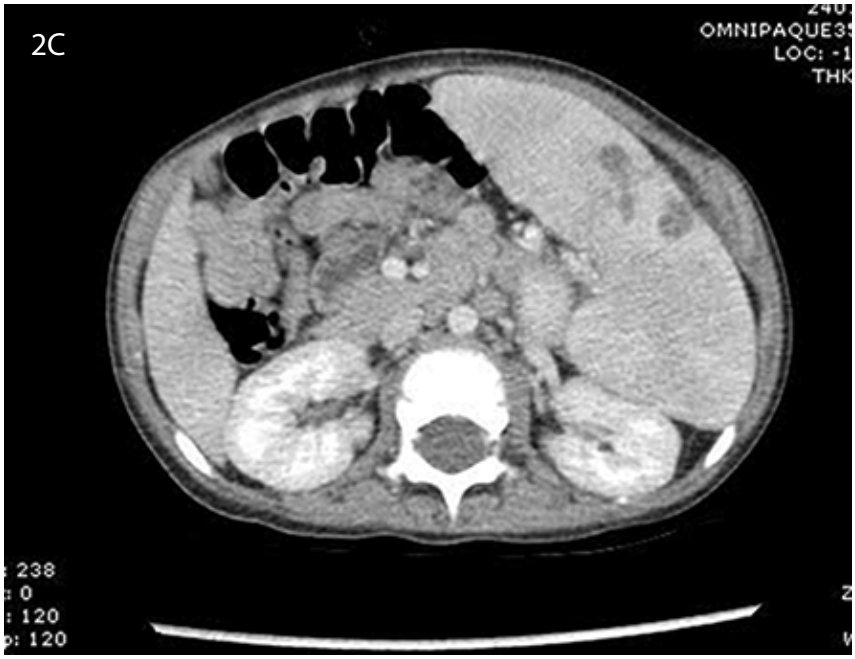

Figure 2. (Continued) (C) CT scan showing multiple small, ill-defined, hypodensity lesions on the spleen.

to classic WAS, bone marrow transplantation has been planned for this patient.

Discussion

Although micro-thrombocytopenia is one of the hallmarks of WAS, previous studies reported that XLT[6] and classic WAS[7] patients could have normal-sized or even giant-sized platelets.[9] The current theory regarding the mechanism of thrombocytopenia in WAS patients is explained by increased destruction of platelets in the spleen.[10] However, the mechanism of micro-thrombocytopenia in WAS remains poorly understood. One recent study proposed that micro-thrombocytes might develop from the presence of increased platelet-derived icro -particles in WAS patients.[11] When compared to the previous tudies which reported patients with normal MPV,[6,7,9,11] our patient had a mutation in the same exon reported by Skoric, *et al.*,[9] but a different exon compared to the other 2 reports (exons 7, 9).[6,7] Thus the WAS diagnosis should consider even when patients have normal MPV, and there are no genotype -phenotype correlation.

Based on our review of the literature, this is the second reported case of WAS with BCG infection. The first case was a Japanese boy who developed fever, ulcers at the BCG vaccinating site, tuberculid-like eruptions, left axillary lymphadenitis, ground-glass opacities and nodules in both lungs, and splenic abscesses, one month after BCG vaccination.[8] WAS was diagnosed by the minimal expression of WASp in the lymphocytes and a mutation at intron 11, c.1453þ2T>C.[8] A study in WAS knockout mice showed that they clear BCG more slowly from lung, liver, and spleen.[12] These researchers suggested that the pathogenesis of this defect is related to Th1 CD4+ T-cells rather than macrophages. Because our patient has different mutation from the Japanese boy, this BCG infection cannot be explained by genotype-phenotype correlation. The rare report of BCG infection in WAS might be explained by the fact that BCG vaccine are not given routinely worldwide, especially in developed countries.

Splenomegaly in WAS patients is rarely reported. Our patient had multiple splenic nodules resulting from *M. bovis* infection. The granulomatous infection of the spleen involving the splenic hilum caused compression of the splenic vein outflow. Accordingly precipitated splenomegaly and back flow pressure via short gastric vessels to the stomach causing severe gastric varices.

Autoimmune diseases have been reported in as many as 40% of patients with WAS.[13] The molecular mechanisms underlying the increased incidence of autoimmunity in WAS can be explained by a defective function of regulatory T cells. The manifestations are markedly different from autoimmune diseases in the general population.

Autoimmune hemolytic anemia (AIHA) and vasculitis are the two most common autoimmune disorders in WAS patients, respectively.[13] Our patient developed AIHA and skin lesion with suspected vasculitis when he was 23 months old.

Molecular analysis of WAS gene from previous studies showed that missense mutation is the most common and is predominantly located at the N-terminus (exons 1-4); whereas, nonsense, insertion, deletion, and splice-site mutations most often occur at the C-terminal region (exons 6-11) of the expressed protein.[2,14-17] In our proband, molecular analysis identified a hemizygous 13-bp deletion (c.181_193delGCT GAGCACTGGA) in exon 2 of the *WAS* gene, which is an uncommon location for a deletion. This mutation is predicted to result in a premature termination at codon 71(p.A61fsX10). To our knowledge, this deletion has never been reported in the literature and almost all reported deletions have involved less than 10 nucleotides.[1,2,11,14-17]

Unfortunately, WASP expression was not performed in this study. Nevertheless, our patient was assumed to have WASP-negative (absent WASP), given that all deletions in exon

2 of the *WAS* gene were reported to have negative WASP.[1,2] Concerning genotype-phenotype correlation, patients who had negative WASP were more likely to present with WAS phenotype.[1,2] Using a scoring system, our patient initially had a score of 2 (XLT phenotype) at the time of molecular diagnosis. He then progressed to a score of 5 (WAS phenotype) within a year, having developed autoimmune disease. This progression confirms the unreliability of the WAS scoring system in pediatric patients aged less than 2 years.

The genetic analysis of the mother was performed and revealed heterozygosity for the same mutation. Thus, our proband's mutation was inherited from his carrier mother. This information facilitates appropriate genetics counseling to the family.

Conclusions

In males with persistent thrombocytopenia, a high index of suspicion for XLT/WAS is recommended. The WAS scoring system can progress over time, especially in children less than 2 years of age. BCG infection can be found in WAS. Therefore, careful physical examination and investigation are needed, especially before starting the patient on immunosuppressive drugs and performing bone marrow transplantation.

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