



The First Case of BENTA Disease (B Cell Expansion with NF-κB and T Cell Anergy) from Iran

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Here we report the first case of BENTA (B cell expansion with NF-κB and T cell anergy) disease from Iran, a 12-year-old girl with non-consanguineous parents and no family history of the disease. BENTA disease is a rare primary immune deficiency that was classified by the 2015 International Union of Immunological Societies (IUIS) under “predominantly antibody deficiencies.” The disease is caused by CARD11 gene gain of function (GOF) mutations and results in constitutive NF-κB activation in B and T cells [1]. This patient was referred to us at 11 years of age as an undiagnosed case, for whole-exome sequencing (WES). A known G123S (Chr7-2,984,163, c.367G>A, p.Gly123Ser) GOF mutation in the CARD11 gene, in the heterozygous state, was identified by WES in DNA extracted from her peripheral blood. Sanger sequencing confirmed the result and showed the absence of this mutation in her parents (supplementary Figure 1). The G123S germline mutation was first reported by Snow et al., in 2012 in an adapted 6-year-old girl from China [2] and later in two other patients from Europe and India [3, 4]. Clinical presentations of all BENTA disease patients include early-onset polyclonal B cell proliferation leading to peripheral blood lymphocytosis, splenomegaly, lymphadenopathy, recurrent upper respiratory tract infections, and susceptibility to other types of viral infections including Epstein–Barr virus

(EBV) [5]. Autoimmunity with various severities has also been reported in some patients including two of the previously reported ones with G123S mutation [2, 4, 5].

Our proband presented splenomegaly, frequent respiratory tract infections, cervical lymphadenopathy, and tonsillitis since birth. Her splenomegaly was diagnosed when she was 18 months old. By age 11, her spleen was 210×120×50 mm, with accessory spleen measuring 12×10 mm, seen adjacent to the splenic hilum. She also presented non-immune-related persistent anemia and thrombocytopenia and had an event of RBC and platelet transfusion during a tonsillitis crisis 2 years ago. Bone marrow aspiration at 3 years of age showed normal cellularity and M/E ratio of 2/1, normal myelopoiesis, normal megakaryocytes, erythroid hyperplasia, and a mild lymphocytic infiltration (30–35%) with no parasitic infection and no increase of blast cells. Morphological examination of her peripheral blood at the same time showed anisopoikilocytosis, mild hypochromia, mild elyptocytosis, normal WBC, and normal platelets. Iron deficiency, alpha and beta thalassemia, G6PD deficiency, and autoimmune hemolytic anemia were ruled out prior to this investigation.

Splenomegaly and frequent upper respiratory tract infections observed in our patient were among characteristic phenotypes observed in all BENTA patients. However, what makes this case interesting is that contrary to other reported BENTA cases including those with the same G123S mutation [2–5], our patient did not show a persistent “absolute lymphocytosis,” the most characteristic phenotype of BENTA disease. Instead, investigating her 12-year hematological data (Fig. 1 and supplementary Table 1) showed a persistent “relative lymphocytosis” with the percentage of neutrophils seldom reaching up to 40%. She only presented a short episode of absolute lymphocytosis at 1st and 2nd years of her life with absolute lymphocyte counts (ALC) of $14.7 \times 10^3/\mu\text{l}$ and $10.7 \times 10^3/\mu\text{l}$ respectively. This phenotype was replaced in subsequent years by normal or low absolute lymphocyte count and frequent episodes of absolute neutropenia and leucopenia, which increased in severity by age over the next 10 years (Fig.

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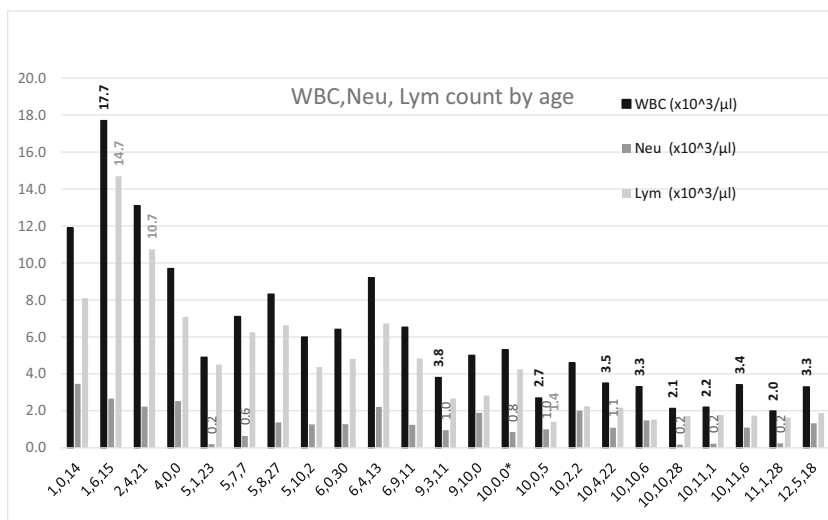
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Fig. 1 The absolute white blood cell (WBC), neutrophil (Neu), and lymphocyte (Lym) counts of the patient from the past 12 years, by age. In the horizontal age axis, the age of the individual is presented by three numbers as the number of years, the number of months, and the number of days. *The day before hospitalization due to a tonsillitis crisis and receiving RBC and platelet transfusions. Only the numbers representing data out of reference intervals for each bar are represented. Supplementary Table 1 contains complete data



1 and supplementary Table 1). She also presented persistent thrombocytopenia, which started in the 5th year of her life. Unlike the previously reported cases with G123S mutation [2–4], our patient had no history of EBV infection and was negative for EBV in a recent antibody test (anti-EBV IgG (VCA) < 10.0 U/ml; anti-EBV IgM (VCA): negative). Other antibody test results were as follows: IgG: 501 mg/dl; IgM: 37 mg/dl; IgA: 169 mg/dl; IgE: < 1 IU/ml; anti-HIV: negative; HBs Ag: negative; HBs Ab < 3.00 mIU/ml; HBc Ab (IgM): negative; HBc Ab (IgG): negative; HBe Ab: negative; anti-HCV: negative.

In the first G123S patient reported by Snow et al., a mild autoimmune neutropenia with anti-neutrophil antibodies along with persistent thrombocytopenia, which was thought to be secondary to splenic sequestration has been reported [2]. In the G123S patient reported by Gupta et al., severe autoimmune hemolytic anemia along with autoimmune thrombocytopenia and hemophagocytosis at later stages of illness has been reported [4]. No evidence of autoimmunity was detected in our patient. Autoimmunity was not also present in the case presented by Outinen et al., with G123S mutation [3]. Our case presented persistent microcytic hypochromic anemia, which seemed to be improving by age. Only during a tonsillitis crisis at 10 years of age, she received RBC together with platelet transfusion due to very low hemoglobin and platelet levels. However, her Coombs direct and indirect tests were negative. Whether the persistent thrombocytopenia and neutropenia in this patient is related to bone marrow deficiency have autoimmune nature or secondary to other manifestations of BENTA disease remains to be determined by further investigations.

Immunophenotyping of the patient's peripheral blood at 11 years of age by flow cytometry showed a population of mononuclear cells (about 81% of the cells analyzed were in the lymphocyte region), a leukopenia with WBC concentration of $2.20 \times 10^9/L$ (RI: 5.00–13.00) and normal ALC of $1.78 \times 10^9/L$ (RI: 1.00–5.00). Moreover, the CD10+ value in this

patient was < 1%. These findings were in contrast to absolute lymphocytosis and excess of immature B cells observed in previously reported BENTA cases including those with the same mutation [2, 4, 5]. However, similar to previously reported BENTA patients, our case had a higher than normal percentage of CD19 (51.35%) (RI: 13–27). Other findings that we found hard to interpret were CD5+CD19+ dual-positive cells of 24.24% and CD19+CD38+ dual-positive cells of 47.23% (supplementary Figure 2).

Furthermore, similar to other BENTA patients including those with G123S mutation [2–4], our case presented an increase in double-negative T (DNT) cells (CD45+CD3+: 40.15%, CD3+CD4-CD8-DNT TCR $\alpha\beta$ + in total lymphocytes: 2.29%, and CD3+CD4-CD8-DNT TCR $\alpha\beta$ + in CD3+ lymphocytes: 5.71%) (supplementary Figure 3). Elevated DNT cells are an overlapping phenotype between autoimmune lymphoproliferative syndrome (ALPS) and BENTA patients, which could lead to misdiagnosis [4]. However, identification of a known mutation in the CARD11 gene associated with BENTA disease, in the absence of any ALPS-related significant variant in our WES results, strongly supported the diagnosis of BENTA disease in this individual.

Currently and during the past 9 years, our patient has used antihistamine (cetirizine) treatment in view of clinical suspicion of allergy as the source of her frequent upper respiratory tract infections. She has been treated with antibiotics during her frequent infection crisis. Hand-wrist radiography of the 11-year-old showed a bone age of 7 years and 10 months. To improve her immunity and growth, she receives oral vitamin supplements on daily basis. Currently, she is in good health and suffers less frequent upper respiratory tract infection crisis during a quarantine at home period started since the COVID-19 pandemic.

Due to a lack of experience about BENTA disease, this case would not be diagnosed without performing WES. Close monitoring for malignant, autoimmune,

and infectious complications are necessary for patients with BENTA disease. This includes assessment of monoclonal B cell expansion by flow cytometry and immunoglobulin heavy chain analysis. Finding strategies to control complications and better management of BENTA disease is an area of research and therapeutic potential of drugs that target CARD11–BCL10–MALT1 (CBM) signaling pathway such as MALT1 protease inhibitors are under investigation [5].

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10875-021-00965-0>.

Authors' Contributions M. Neishabury obtained the research grant, supervised the experimental work, analysed the data, and wrote the manuscript. M. Mehri performed the experimental work and contributed to WES data analysis. H. Najmabadi was the molecular diagnostics advisor. T. Cheraghi and A. Azarkeivan were the physicians in charge of the patient and contributed to manuscript revision.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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