

Letter to the Editor

Timely follow-up of a GATA2 deficiency patient allows successful treatment

To the Editor:

GATA binding protein 2 (GATA2) deficiency is a complex disorder of hematopoiesis characterized by a large spectrum of clinical manifestations, which cause a deficiency of dendritic cells, monocytes, B cells, and natural killer cells. A number of GATA2-deficient clinical syndromes have been described: monocytopenia and *Mycobacterium avium* complex infection syndrome, familial myelodysplasia (MDS)/acute myeloid leukemia (AML), lymphedema, deafness, and myelodysplasia (Emberger syndrome).¹ The disease is caused by GATA2 gene mutations, and it typically presents in later childhood or adulthood.² GATA2 is a member of the GATA transcription factor family that plays a role in the regulation of hematopoiesis; in particular, it is necessary for the maintenance and survival of the hematopoietic stem cell pool.³ Patients are susceptible to mycobacterial, viral, and fungal infections and show a predisposition to develop myelodysplasia, acute or chronic leukemias, lymphedema, and pulmonary alveolar proteinosis (PAP). Early diagnosis is critical to direct clinical management, preventive care, and family screening.

We report the case of a child coming to our attention because of recurrent episodes of aphthous stomatitis, warts, and leukopenia. The patient was the second child of nonconsanguineous parents. Neither the parents nor the elder brother had a history of increased susceptibility to infection. Medical history revealed a healthy childhood despite recurrent episodes of otitis and pharyngitis early in infancy, which improved after adenoidectomy at age 4 years. At the same age, he also had an uneventful chickenpox. From the age of 6 years, our patient began to suffer from recurrent episodes of aphthous stomatitis every 2 to 3 months, sometimes associated with fever and minor episodes of warts. At the age of 7 years, he was referred to our unit because of mild leukopenia and neutropenia (white blood cells, 3300/ μ L; neutrophils, 877/ μ L) revealed by complete blood cell count with differential. We confirmed leukopenia and neutropenia with absence of monocytes. The clinical examination was unremarkable except for a small area of alopecia on his head. Cytomegalovirus, EBV, and Toxoplasma infections were excluded. Celiac disease screening was negative. Organ-specific and nonorgan-specific autoantibodies were all negative. Antineutrophil antibodies were negative in 4 different blood samples. Immunologic studies showed serum immunoglobulins within normal range. Lymphocyte subset evaluation by flow cytometry showed B cells at the lower limits of normal range (240/ μ L), normal T cells (CD3+, 2310/ μ L; CD3+CD4+, 1270/

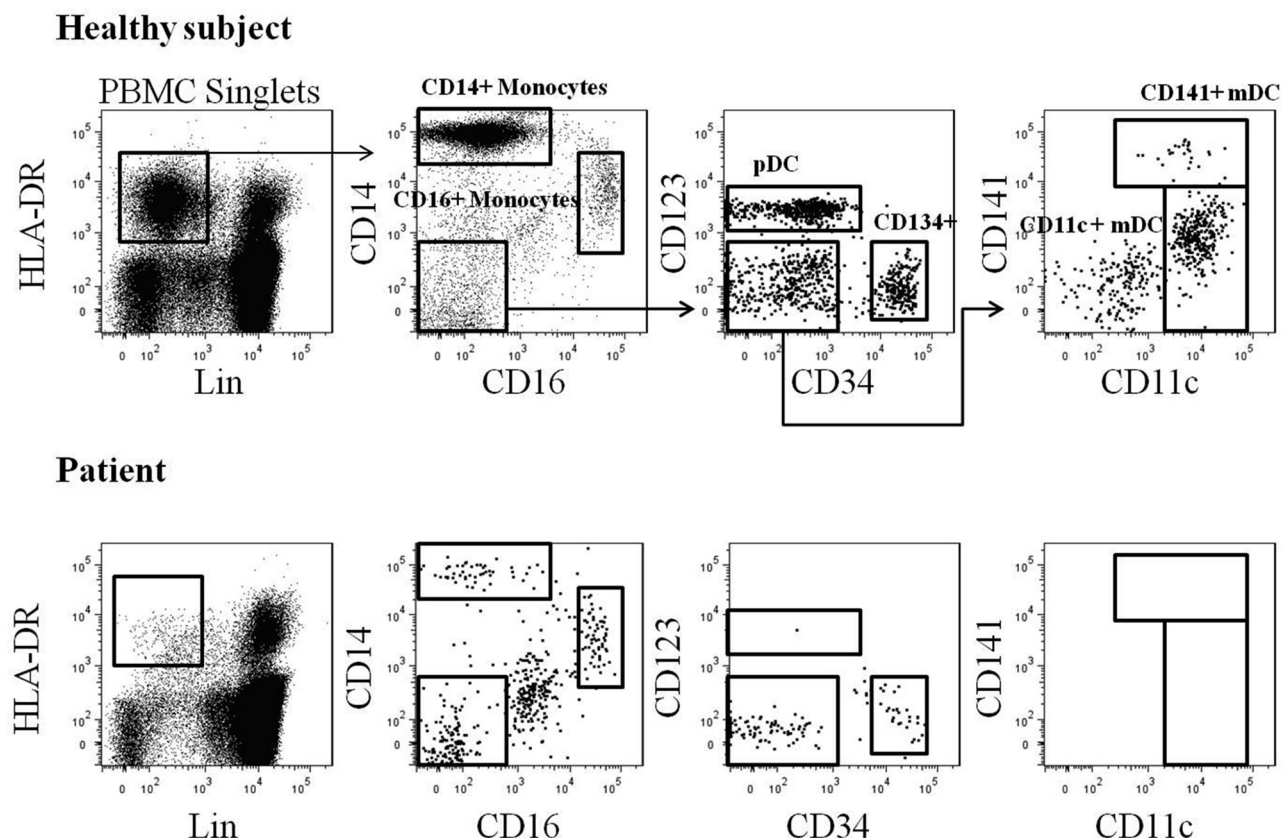


FIG 1. Flow cytometry analysis of peripheral blood cells showing the identification of monocyte and dendritic cell subsets in a patient and a healthy subject. Gating strategy and cell populations are shown in the healthy subject dot plots. The absence of pDC and mDC and decrease in monocytes are evident in the patient compared with the healthy subject. mDC, Myeloid dendritic cell; pDC, plasmacytoid dendritic cell.

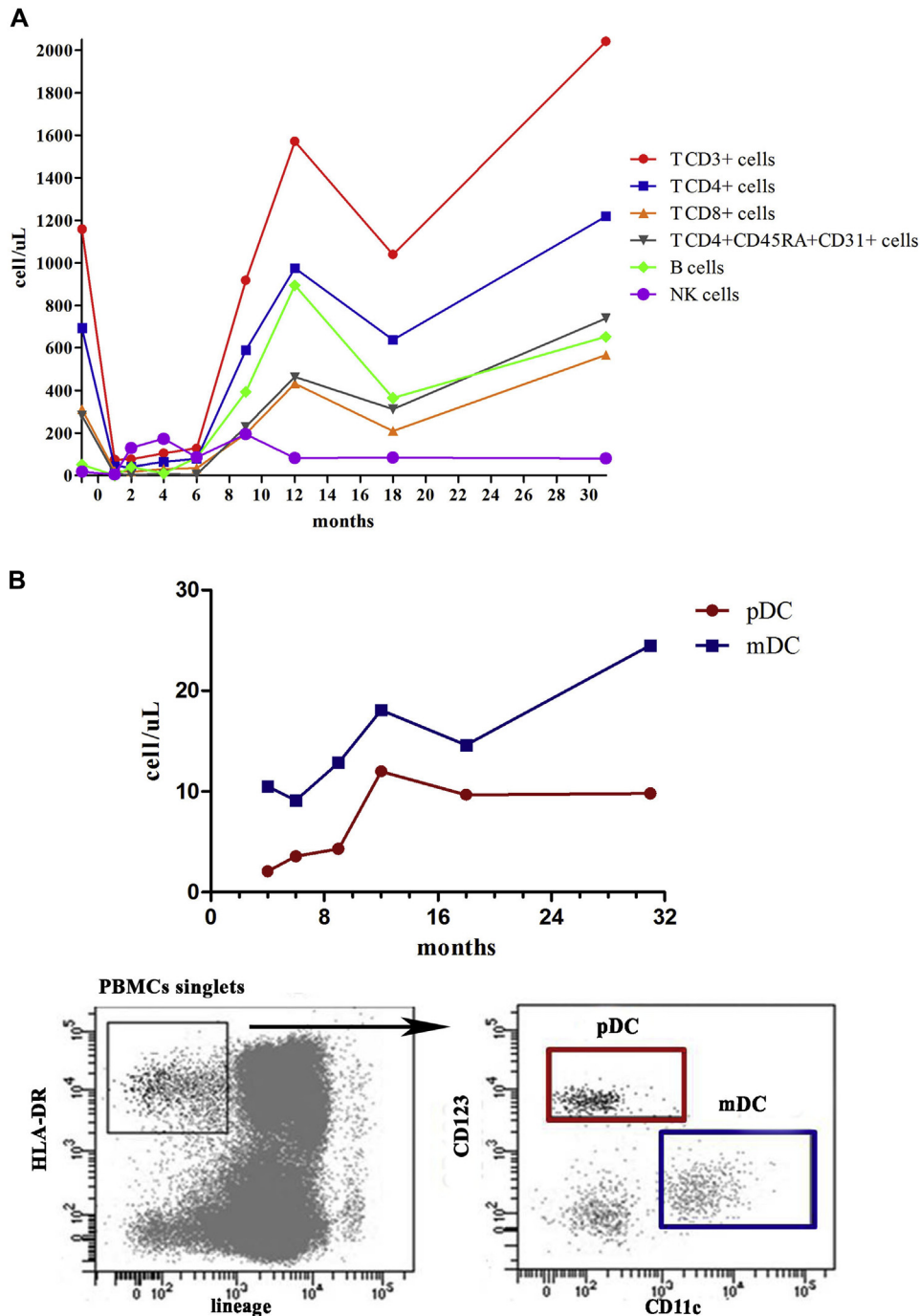


FIG 2. Immune reconstitution after HSCT. Time zero (x axis) represents the date of HSCT. **A**, Absolute count of T, B, and NK cells (per μ L of blood) in peripheral blood pretransplant and posttransplant. **B**, Dendritic cell immune reconstitution at different times posttransplant. The increase in mDC and pDC subpopulations is evident. Gating strategy for the identification of human pDC and mDC is shown in the lower panel. *HSCT*, Hematopoietic stem cell transplantation; *mDC*, myeloid dendritic cell; *NK*, natural killer; *pDC*, plasmacytoid dendritic cell.

μ L; CD3+CD8+, 580/ μ L), and low natural killer cells (110/ μ L). Double-negative T cells were within normal range (CD3+TCR $\alpha\beta$ +CD4-CD8-: 1% of CD3+lymphocytes).

The patient underwent bone marrow aspirate that revealed slight hypocellularity given his age, with mild signs of dysmyelopoiesis. Molecular analysis of the *TERC* and *TINF2* genes did not reveal any mutations. Chromosomes 7, 8 LSI 21 (21q22.13-q22.2), and

fluorescence *in situ* hybridization (FISH) analysis were negative. The investigations undertaken allowed us to exclude congenital or autoimmune neutropenia, rheumatological diseases (ie, systemic lupus erythematosus), autoimmune lymphoproliferative syndrome, antibody defects, combined immunodeficiencies, or bone marrow failure (see Table E1 in this article's Online Repository at www.jacionline.org). Shortly after our initial evaluation, he suffered

from Parvovirus B19 infection (virus detected with PCR in blood and bone marrow) characterized by high fever and arthritis. He rapidly recovered from the symptoms, but had difficulty in clearing the virus because it was detected for months in the bone marrow without involvement of peripheral hematopoietic lineages. Nonetheless, during the follow-up, he was clinically well with normal growth.

Despite the atypical clinical phenotype, the absence of monocytes prompted us to hypothesize that the patient might have sporadic dendritic cell, monocyte, and lymphocyte deficiency and to request molecular investigation of the *GATA2* gene and the dosage of Fms-like tyrosine kinase 3 ligand serum levels. Myeloid dendritic cells and plasmacytoid dendritic cells were undetectable in peripheral blood (Fig 1), and elevated Fms-like tyrosine kinase 3 ligand serum levels (8452 pg/mL; healthy subject, 10 pg/mL; normal range, 48.3-173 pg/mL) were found. *GATA2* sequencing revealed a mutation in exon 3 (c.257_258delGC; p.C85fs), which results in a truncated protein. Further sequencing of gDNA from his healthy family members revealed no mutations. Ultimately, the patient was diagnosed with *GATA2* deficiency with a *de novo* *GATA2* mutation, despite the absence of typical clinical monocytopenia and *Mycobacterium avium* complex infection/Emberger syndrome, or familial MDS/AML manifestations.

Given the high risk of progression to MDS in patients with *GATA2* deficiency, we monitored this patient very carefully, checking his bone marrow every 6 months. After 1 year, he developed refractory cytopenia according to WHO 2008 ICD-099853 (see Fig E1 in this article's Online Repository at www.jacionline.org). This was confirmed at 1 and 3 months of follow-up, and the FISH analysis performed on a new bone marrow aspirate revealed the presence of chromosome 7 monosomy in 30% of investigated nuclei.

Therefore, because of the onset of MDS, at age 9 years, he underwent hematopoietic stem cell transplant from his HLA-matched (*GATA2* wild-type) brother. He received treosulfan, fludarabine, and thiopeta as conditioning and methotrexate and cyclosporine as graft-versus-host disease prophylaxis posttransplantation (see Fig E2 in this article's Online Repository at www.jacionline.org). The regimen chosen was intended to enable adequate myeloablation with limited toxicity. He was engrafted on day 23 and developed low-grade intestinal graft-versus-host disease, which was successfully treated with steroids. Full donor engraftment was achieved in those hematopoietic compartments deficient pretransplantation as was good immune reconstitution over time (Fig 2) without clinical complications.

The clinical spectrum of *GATA2* deficiency syndrome is somehow unpredictable. Patients' age at onset of symptoms and clinical conditions are variable and include infections in most (60%), MDS/AML in 21%, cytopenias, lymphedema, and PAP.⁴ The vast majority of cases of *GATA2* deficiency are diagnosed in adolescence to early adulthood when a more defined clinical phenotype appears. Cases with earlier onset (childhood) are generally reported within *GATA2*-mutated families where other older members were previously diagnosed.⁵⁻⁷

Hematopoietic stem cell transplant is the unique curative therapy available at the moment, although there are no clear indications in the literature of the best conditioning regimen and timing to perform transplantation.⁸

Our patient is the youngest index case of *GATA2* deficiency reported thus far, with no other family member being affected, and was diagnosed despite an atypical clinical phenotype. At the

time of diagnosis he did not suffer from major symptoms and had no signs of infections and/or MDS. We carefully followed him, monitoring his bone marrow every 6 months and, given the HLA identical sibling donor, we decided to start the transplant program as soon as his hematological situation progressed toward MDS. The early detection of MDS allowed timely HSCT before the development of AML or other *GATA2*-associated life-threatening complications such as PAP, end-organ damage from autoimmunity, or other malignancies. The patient is now 3 years posttransplant with full donor engraftment and clinically well.

The constantly increasing awareness of *GATA2* deficiency should help physicians to achieve an early diagnosis. The reported case is an interesting example of how clinical practice should be supported by timely therapeutic intervention. Hematopoietic stem cell transplantation reverses most of the *GATA2*-deficient clinical phenotypes with good long-term outcomes.

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Sara Ciullini Mannurita, MSc^{a,*}

Marina Vignoli, PhD^{a,*}

Gloria Colarusso, MD, PhD^a

Fabio Tucci, MD^b

Marinella Veltroni, MD^b

Stefano Frenos, MD^b

Veronica Tintori, MD^b

Maurizio Aricò, MD^b

Venetia Bigley, MD, PhD^c

Matthew Collin, MD, PhD^c

Claudio Favre, MD^b

Eleonora Gambineri, MD^{a,b}

From ^athe Department of "NEUROFARBA," Section of Child's Health, University of Florence and ^bthe Hematology-Oncology Department, "Anna Meyer Children's Hospital," Florence, Italy; and ^cthe Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom. E-mail: eleonora.gambineri@unifi.it.

*These authors contributed equally to this work.

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METHODS

Immunologic studies

Phenotypic analysis on peripheral blood of monocytes and dendritic cells (monocytoid and plasmacytoid subpopulations) and natural killer cells was performed through staining with anti-CD16, -CD14, -CD56, CD34, -CD123, -CD11c, CD141, -HLA-DR, and -lineage (CD3, CD14, CD16, CD19, CD20, CD56) antibodies (BD Biosciences, Milan, Italy). Moreover, mAbs specific for CD45RA (Beckman Coulter, Brea, Calif), CD3, CD4, CD8, CD31, TCR- $\alpha\beta$, CD19, and CD45 (BD Biosciences) were used to define the percentage of T, B-cell subsets on gated lymphocytes. Flow cytometry data were collected using a FACS Canto II cytometer and analyzed with FACS Diva Software (BD Biosciences).

ELISA

Serum Fms-like tyrosine kinase 3 ligand was measured by ELISA kit according to manufacturers' instruction (Quantikine human Flt3/Flk-2 Ligand Immunoassay, R&D Systems, Minneapolis, Minn).

Sequence analysis

Genomic DNA (gDNA) was isolated from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The *GATA-2* coding sequence including the exon-intron junctions, was amplified from gDNA by PCR with specific primer pairs that are flanking the regions of interest, using a 2700 thermal cycler (Thermo Fisher Scientific, Carlsbad, Calif). Primer pair sequences were already reported in the literature.^{E1}

Approximately 50 ng of genomic DNA was amplified with the following conditions: 94°C 4 minutes, 35 cycles at 94°C 30 seconds, 30 seconds at a primer-specific melting temperature and 68°C for 30 seconds, with a final extension at 68°C for 5 minutes. PCR products were purified using the ExoSAP-IT reagent (USB and Affimetrix, Cleveland, Ohio), sequenced by using the BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific) on an automated 3130xl Genetic Analyzer (Thermo Fisher Scientific) and sequencing analysis was carried out with BioEdit sequence alignment software (Ibis Therapeutics, Carlsbad, Calif).

Chimerism analysis

Chimerism was evaluated by multiple fluorescent short tandem repeat analysis using AmpFISTR Identifier amplification kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The tetranucleotide STR loci amplified in this reaction included D8S1179, D21S11, D7S820, and CSF1PO (all labeled with 6-Fluorescein blue dye); D3S1358, TH01, D13S317, D16S539, and D2S1338 (all labeled with VIC green dye); D19S3433, vWA, TPOX, and D18S51 (all labeled with NED yellow dye); and D5S818 and FGA (all labeled with PET red dye). In addition, the amelogenin locus was analyzed to differentiate X and Y chromosomes (labeled with PET red dye). The PCR products were analyzed with an ABI PRISM 310 DNA Genetic Analyzer (Thermo Fisher Scientific). Fragment size and peak area data were determined by GeneScan software (Thermo Fisher Scientific). The degree of chimerism was calculated using the method described elsewhere.^{E2}

FISH

FISH analyses were carried out by the national reference laboratories according to standard procedures to detect -7 or 7q- on bone marrow-fixed cells. Cytogenetic data were described according to International System for Human Cytogenetic Nomenclature 2009.^{E3}

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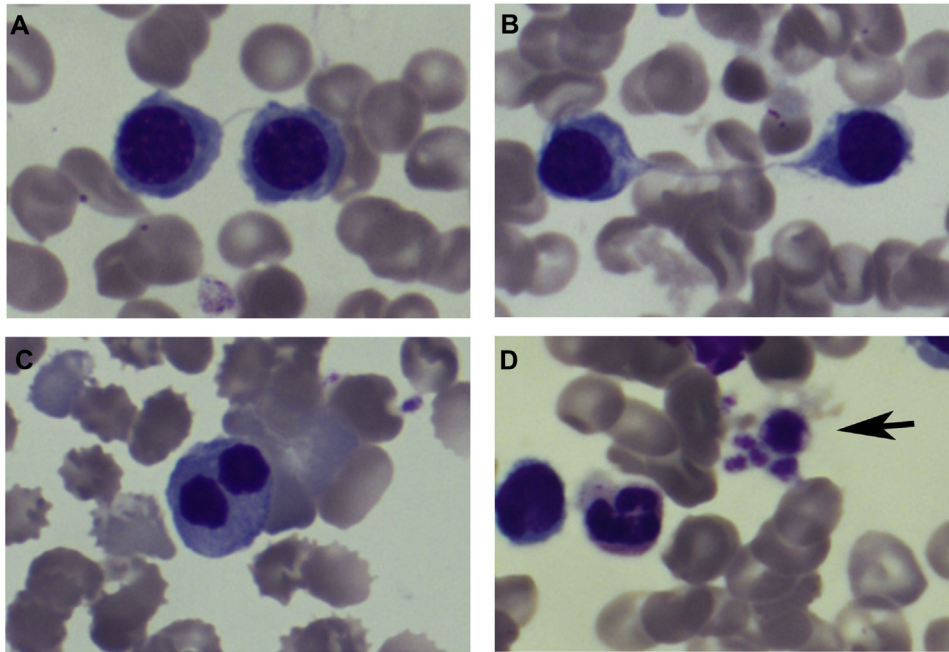


FIG E1. Bone marrow aspirate smear stained with May-Grünwald Giemsa (original magnification $\times 100$). Presence of cytoplasmic bridges between 2 erythroblasts (**A** and **B**), binuclear erythroblast (**C**), giant platelets (*arrow*) (**D**).

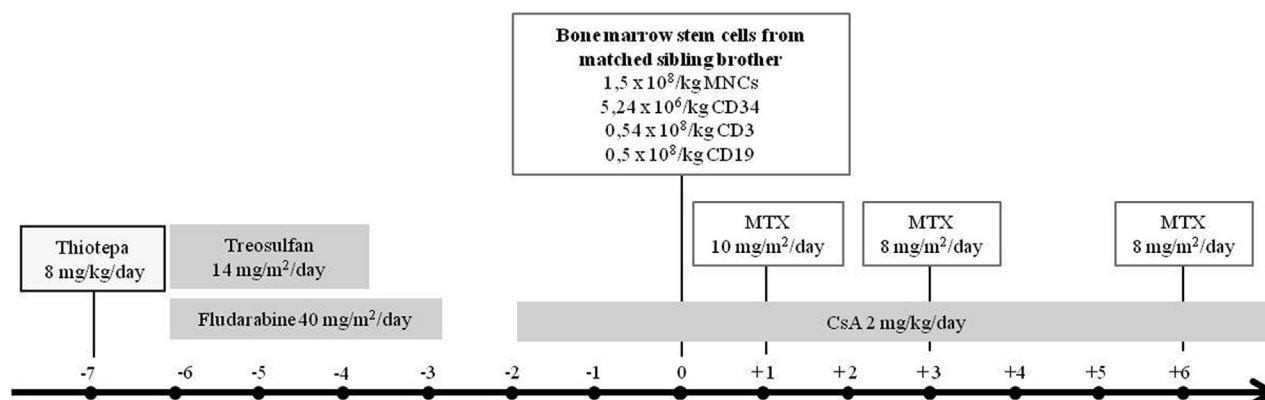


FIG E2. Timeline representing HSCT conditioning and GvHD prophylaxis. The numbered line indicates the time (days) before and after transplantation. Day zero is the day of graft infusion. *CsA*, Cyclosporin A; *GvHD*, graft-versus-host disease; *HSCT*, hematopoietic stem cell transplantation; *MNC*, mononuclear cells; *MTX*, methotrexate.

TABLE E1. Summary of clinical and laboratory findings, possible differential diagnosis, and indication for performing HSCT

Clinical symptoms	Laboratory findings	Differential diagnosis	HSCT indication
UTI	Mild leukopenia and neutropenia	Congenital/autoimmune neutropenia	Chromosome 7 monosomy
Aphthous stomatitis	Absent monocytes and DCs Low B cells/NK cells	Rheumatological diseases (ie, SLE)	
Warts	Bone marrow hypocellularity with mild dysmyelopoiesis	ALPS	
Fever and reactive arthritis (Parvovirus B19 infection)	Normal immunoglobulin values Negative autoimmune screening	Bone marrow failure (TERC/TINF2)	
Localized alopecia areata	Negative viral PCRs	Unspecified antibody defects (but normal serum immunoglobulin levels)	

See text for more details.

ALPS, Autoimmune lymphoproliferative syndrome; SLE, systemic lupus erythematosus, UTI, upper respiratory tract infection.