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Case reports in pediatric hematology and hematological malignancies 2022

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Case reports in pediatric hematology and hematological malignancies 2022

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Editorial: Case reports in pediatric hematology and hematological malignancies 2022

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Editorial on the Research Topic
Case reports in pediatric hematology and hematological malignancies 2022

Case reports on pediatric hematology and hematologic malignancies are featured in this issue. Oncohematology and pediatric hematology are poorly characterized in the scientific literature, particularly in case reports. This type of information, based on professional experience with clinical cases, is very relevant for clinical practice because it enables the professional who attends to this group of patients to adequately clarify and manage these cases, bringing the appropriate resolution to many cases and improving the quality of life of these patients.

The case described by Thalji et al. included a 4-year-old male boy who had a blueberry muffin rash on his face and neck but had no additional systemic involvement. A biopsy revealed that the patient had congenital cutaneous LCH. This early detection shields patients from needless and potentially harmful systemic treatment (Thalji et al.).

The case reported by Wang et al. reported a 13-year-old male child with recurrent epistaxis for 6 months, anemia for 1 month, and a chest CT scan compatible with hemorrhagic foci was given in the second case report. He was diagnosed with acquired von Willebrand syndrome, had no family history, and tests revealed that he had antinuclear and anti-Sm antibodies, indicating a link to Systemic Lupus Erythematosus (Wang et al.).

The third case, described by Jenei et al., is a 5-year-old male child diagnosed with interdigitating dendritic cell sarcoma during treatment for B-cell precursor acute lymphoblastic leukemia, according to another clinical case described in this issue. Multiple lymph nodes in the cervical chain were discovered in this patient, who was detected with positron emission tomography CT, treated with chemotherapy, and then had bone infiltration 4 years later. With this condition, an allogeneic hematopoietic stem cell transplant was conducted successfully (Jenei et al.).

Zhou et al. described a 6-year-old girl who had a clinical manifestation of leukemia cutis with a 10-month history of presentation with cutaneous plaques and abscesses as furuncles. The initial clinical indications of Chronic Neutrophil Leukemia BCR-ABL Negative

Myeloproliferative Neoplasm were these cutaneous appearances. Successful allogeneic hematopoietic stem cell transplantation was also used to treat the patient. This discovery was pertinent to dermatologists (Zhou et al.).

A report of Hannemann et al. highlighted a major but rare complications of allogeneic hematopoietic cell transplantation (HCT): an invasive fungal infection with fusariosis as a lethal infections despite neutrophil engraftment and uninterrupted treatment with voriconazole. Unfortunately, this 16-year old patient with severe aplastic anemia developed splenic rupture and endocarditis. He also developed embolic cerebral infarctions with unilateral hemiparesis, and despite cardiac surgery, he did not regain consciousness because of diffuse cerebral ischemia and died on day +92 post HCT. Disseminated infection with fusarium solani is a rare complications post allogeneic HCT with poor outcomes (Hannemann et al.).

The case presented by Krstic et al. described the first case of T-cell acute lymphoblastic leukemia (T-ALL) with CCDC88C: JAK2 fusion, with normal karyotype, detected by whole genome sequencing. The patient fortunately, responded to nelarabine and intensive chemotherapy and so proceeded to allogeneic HCT. The patient is now in remission 8 months post HCT. High throughput sequencing technologies have shown enormous potential in the genetic characterization of hematological malignancies, particularly in pediatric ALL, and are paving the way to personalized treatment strategies. Pediatric patients with ALL and genomic features consistent with JAK/STAT pathway activation are currently recruited in ongoing clinical trials for targeted treatment with Ruxolitinib in addition to chemotherapy. However, this report serves as an example of the power of WGS in the diagnostic setting of acute leukemia, as it enables timely recognition of potential therapeutic targets in high-risk pediatric ALL (Krstic et al.).

The case presented by Ipe et al., reported a 12-year old patient with B-ALL who relapsed 15 months after diagnosis while receiving maintenance chemotherapy. For his relapsed disease, he underwent CAR-T cell therapy to avoid allogeneic HCT and risk of DNA damage with radiation due to lately discovered germline heterozygous checkpoint kinase 2 (CHEK2) mutation. This mutation was discovered when the patient presented with papillary thyroid carcinoma 7 months after initial diagnosis and before relapse of his B-ALL. The patients remains in remission 3 years after CAR-T cell therapy. CHEK2 is a tumor suppressor gene that plays a critical role in the cell cycle and response to DNA damage induced by replication stress. Whether the CHEK2 gene is a true cancer predisposition syndrome gene remains a topic of debate especially in relation to acute leukemia. More research in delineating the true role of pathogenic CHEK2 gene mutations in hematologic malignancies as a potential cancer predisposition gene is needed (Ipe et al.).

In the report of Leonardi et al., a patient with a long and really challenging clinical history is reported. Kabuki syndrome (KS) is a rare multisystemic disease due to mutations in the *KMT2D* or *KDM6A* genes, which act as epigenetic modulators of different processes, including immune response. The syndrome is characterized by anomalies in multiple organ systems, and it is associated with autoimmune and inflammatory disorders, and an underlying immunological phenotype characterized by immunodeficiency and immune dysregulation. This case emphasizes the importance of suspecting immune dysregulation in KS (Leonardi et al.).

In the report of Naviglio et al., a case of treatment with venetoclax and azacitidine in a patient with AML in Shwachman-Diamond syndrome is reported. BCL-2 inhibitor venetoclax has revolutionized the treatment of AML in elderly adults, especially for treatment-naive elderly patients who are ineligible for intensive chemotherapy and there is limited evidence on the use of venetoclax in pediatric patients with SDS-related MDS or AML. This represents an experience suggestisting the potential role of the combination venetoclax/azacitidine for patients with SDS and AML, whose safety and efficacy need to be evaluated in a clinical experimental model (Naviglio et al.).

In the report of Akiely et al. the first pediatric case of myeloid/lymphoid neoplasm with PDGFRA rearrangement presenting with synchronous myeloproliferative disease and B-LBL. The patient was started on imatinib with concomitant therapy for B-LBL per the Children Oncology Group (COG) standard therapy for localized B-LBL and demonstrated a favorable outcome in the 2. 5-year follow-up period (Akiely et al.).

In this report Guerra et al. describe a rare case of patients with pediatric myelofibrosis, showing different clinical and pathological features when compared to the WHO 2016 Primary Myelofibrosis classification. They aslo retrospectively collected and analyzed 14 consecutive pediatric myelofibrosis, classified into three subgroups: adult-like myelofibrosis, pediatric immune myelofibrosis, idiopathic myelofibrosis. Pediatric Immune Myelofibrosis was the predominant subgroup in our cohort (7/14). Pediatric Immune Myelofibrosis is characterized by peculiar bone marrow features (i.e., T lymphocyte infiltration) and a milder course compared to the other patients Pediatric Immune Myelofibrosis is a novel and distinct pathological entity (Guerra et al.).

In the report Eguchi et al. describe 10-month-old infant with an extremely rare combination of Juvenile Myelomonocytic Leukemia and Ornithine Transcarbamylase Deficiency Safely. One noteworthy aspect of the present case is the proven involvement of congenital UCD in the pathogenesis of hyperammonemia associated with cancer treatment. The treatment of the patient was complicated by occurrence of severe hyperammonia. Authors underline that hyperammonemia should be considered a differential diagnosis when unexplained and non-specific symptoms occur during the treatment of hematologic malignancies (Eguchi et al.).

In the report of Saettini et al. two patients with identical germline *CBL* mutation and clinical and immune-hematological overlapping features with autoimmune lymphoproliferative syndrome (ALPS) and B-cell expansion with NF-κB and T-cell anergy (BENTA) syndrome are described. CBL syndrome is a

Noonan-like RASopathy with heterogeneous clinical phenotype and predisposition to juvenile myelomonocytic leukemia (JMML). Although the phenotype of children affected by CBL syndrome has been increasingly delineated over time, the immunological features of CBL syndrome have not been extensively described (Saettini et al.).

We really hope that reading this issue, which contains these rare clinical situations in pediatrics, hematology, and oncohematology, would be beneficial to professional knowledge and clinical care for this population.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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Case Report: Juvenile Myelomonocytic Leukemia Underlying Ornithine Transcarbamylase Deficiency Safely Treated Using Hematopoietic Stem Cell Transplantation

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Background: Juvenile myelomonocytic leukemia (JMML), which is predominantly found in infants, is a clonal abnormality of pluripotent hematopoietic stem cells and presents with the symptoms of both myeloproliferative tumors and myelodysplastic syndromes. Estimates have shown that ∼20 cases of JMML occur annually in Japan. Ornithine transcarbamylase deficiency (OTCD), the most common among all urea cycle disorders (UCDs), occurs in 1 of 80,000 people in Japan.

Case Presentation: A 10-month-old infant who had fever, vomiting, and diarrhea for 2 days was referred to our hospital for the following abnormalities in blood tests: white blood cell count, 48,200/μL; hemoglobin, 9.0 g/dL; and platelet count, 135,000/μL. Bone marrow examination showed a nucleated cell count of 396,000/mm^3 and blast cell count of 5.0%, as well as decreased mature granulocyte count and slightly myeloperoxidase stain-negative blasts but no monoclonal cell proliferation on May–Giemsa staining. Colony assay showed the proliferation of spontaneous colony and high sensitivity to granulocyte-macrophage colony-stimulating factor. Genetic analysis of peripheral blood mononuclear cells showed that the patient was positive for neuroblastoma RAS (*NRAS*) mutation. The patient was ultimately diagnosed with JMML. Approximately 170 days after his first hematopoietic stem cell transplantation (HSCT), the patient's JMML relapsed. Shortly after the recurrence, nausea, vomiting, hyperventilation, and decreased vitality were observed, followed by a decrease in the level of consciousness. The patient's ammonia level was 472 μmol/L. A test for seven different genetic mutations for the UCD showed the presence of c. 119G>A (amino acid change p. Arg40His). As such, late-onset OTCD was added to his diagnosis. Administration of sodium phenylacetate, l-arginine hydrochloride, and carnitine was continued following the diagnosis of OTCD, after which hyperammonemia was not observed. Regarding JMML relapse, HSCT was performed on day 405 after the first transplantation.

Conclusion: Hyperammonemia should be considered a differential diagnosis when unexplained and non-specific symptoms occur during the treatment of hematologic malignancies. Patients should be tested for UCD as a cause of hyperammonemia, and treatment for hyperammonemia should be continued until the cause is identified. The patient shows normal developmental progress, has an intact neurological status, and has not experienced another hyperammonemia attack. His JMML has remained in remission for over 3 years.

Keywords: juvenile myelomonocytic leukemia, ornithine transcarbamylase deficiency, urea cycle disorder, hyperammonemia, hematopoietic stem cell transplantation, chemotherapy

INTRODUCTION

Juvenile myelomonocytic leukemia (JMML) is a clonal abnormality of pluripotent hematopoietic stem cells, which is predominantly observed in infants and presents with the symptoms of both myeloproliferative tumors and myelodysplastic syndromes (1). Estimates have shown that approximately 20 cases of JMML occur annually in Japan, with the median age at diagnosis being 2 years (2). A suspected case of JMML typically presents with non-specific constitutional signs and symptoms, such as fever, infection, pallor, bleeding, cough, poor weight gain, maculopapular rash, lymphadenopathy, moderate hepatomegaly, marked splenomegaly, leukocytosis, absolute monocytosis, anemia, and thrombocytopenia (3).

Ornithine transcarbamylase deficiency (OTCD) is an X-linked genetic disorder that prevents the breakdown and excretion of ammonia. This allows ammonia to attain toxic levels, which affect the central nervous system (4). OTCD is the most common among all urea cycle disorders (UCDs) (5), and occurs in 1 of 80,000 people in Japan (6, 7). Male neonates with OTCD often experience ammonia toxicity, protein intolerance, and die within a week after birth. However, those with late-onset OTCD (age at onset, ≥28 days) present with a wide range of symptoms that range from asymptomatic phenotype to hyperammonemia, coma, and death (8, 9).

To the best of our knowledge, there has been no report on a case presenting with both JMML and OTCD. Herein, we report the case of an infant boy diagnosed with late-onset OTCD who developed hyperammonemia at relapse after hematopoietic stem cell transplantation (HSCT) for JMML.

CASE PRESENTATION

A 10-month-old infant who had fever, vomiting, and diarrhea for 2 days was referred to our hospital owing to blood test abnormalities. He had no family history of OTCD. He had neither abnormal eating habits nor poor appetite nor insidious symptoms such as liver disorders, behavioral disorders, psychiatric symptoms, growth delay, and developmental delay. His laboratory data were as follows: white blood cell count (WBC), 48,200/μL [normal range (NR): 7,000–15,000/μL]; hemoglobin count, 9.0 g/dL (NR: 13.5–18.0 g/dL); platelet count, 135,000/μL (NR: 150,000–330,000/μL); and C-reactive protein content, 10.3 mg/dL (NR: <0.3 mg/dL). The patient had a height of 69.5 cm [−1.8 standard deviation (SD)] and a weight of 8,800 g (−0.4 SD). His vital signs showed an abnormal body temperature of 39.4°C and a heart rate of 164 beats/min. Physical examination revealed pigmented papules scattered over his extremities, a liver palpable 5 cm in the right hypochondriac region, and a spleen palpable 6 cm in the left hypochondriac region. The initial laboratory tests conducted at our facility showed the following results: WBC count, 60,900/μL; neutrophilic rate, 37.7%; lymphocyte rate, 42.7%; monocyte rate, 19.1% (3–9%); hemoglobin count, 9.1 g/dL; platelet count, 145,000/μL; fetal hemoglobin level, 19.1% (NR: <1.2%); aspartic aminotransferase level, 63 IU/L (NR: 20–45 IU/L); alanine aminotransferase level, 36 IU/L (NR: 4–24 IU/L); gamma-glutamyl transpeptidase level, 40 IU/L (NR: 5–17 IU/L); and ammonia level, 83 μg/dL (NR: <80 μg/dL). Bone marrow examination showed a nucleated cell count of 396,000/mm^3, megakaryocyte count of 0/mm^3, monocyte rate of 5.4%, blast cell count of 5.0%, and myeloid to erythroid ratio of 3.41. It also showed decreased mature granulocytes and count and slightly myeloperoxidase stain-negative blast cells but no monoclonal cell proliferation using May–Giemsa staining (**Figure 1**). Colony assay showed spontaneous colony proliferation and high sensitivity to granulocyte-macrophage colony-stimulating factor. Genetic analysis of peripheral blood mononuclear cells showed that the patient was positive for neuroblastoma RAS (*NRAS*) mutation [Codon 12, 13 mutation-positive c.35G>A (amino acid change p.G12D)]. No germline mutation of *NRAS* was observed. Chromosome analysis showed a normal karyotype. Based on these findings, the patient was diagnosed with JMML.

Figure 2 shows the clinical course of the patient. He had never been treated with any cytoreductive therapy before the first HSCT. After being transferred to an HSCT facility, he was pretreated with busulfan (1.2 mg/m^2 for 4 days), fludarabine (30 mg/m^2 for 4 days), and melphalan (90 mg/m^2 for 2 days) and underwent an allogeneic cord blood transplantation (CBT) 8 months after the diagnosis of JMML. The acute clinical course after CBT was unremarkable, and the complete chimera was confirmed on day 25. On day 66, cytomegalovirus pneumonia was observed; the patient was treated with ganciclovir, and he showed a mild recovery. On day 87, pulmonary hypertension was noted, which was treated with steroids and sildenafil. On day 153,

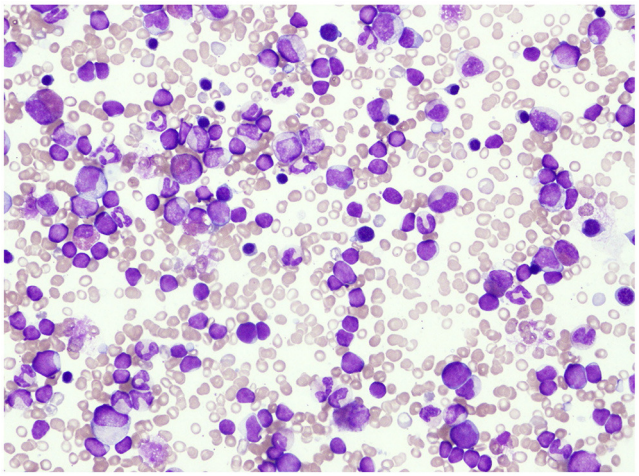

FIGURE 1 | Bone marrow examination showed a decrease in mature granulocytes and slight myeloperoxidase stain-negative blast cells but no monoclonal cell proliferation with May–Giemsa staining.

hypertension was noted, and the patient was subsequently treated with antihypertensive drugs. On day 167, the patient suffered from tubulopathy and nephrotic syndrome, but he showed mild recovery after hyperbaric medicine treatment. Throughout this course, ammonia levels were normal after several measurements.

On approximately day 170, hematopenia (WBC, 2,600/μL; hemoglobin, 11.0 g/dL; and platelet count, 69,000/μL) appeared gradually, and on day 179, a positive peripheral blood mononuclear cell *NRAS* mutation was confirmed, which appeared to have relapsed. Tacrolimus, which was used for managing various complications that could not be ruled out as graft-versus-host disease, was difficult to discontinue. Mercaptopurine hydrate (50 mg/m^2) was then started; however, immediately following administration, nausea, vomiting, hyperventilation, and decreased vitality were observed, followed by a decrease in the level of consciousness. At the start of the medication, his WBC count was 2,300/μL, and chimerism at this time was not confirmed. The ammonia level on the 10th day after the onset of these symptoms was 472 μmol/L and carbon dioxide partial pressure decreased with respiratory alkalosis (pCO_2 23.8 mmHg), which was determined to have been caused by hyperammonemia. Administration of sodium phenylacetate (250 mg/kg), L-arginine hydrochloride (400 mg/kg), and sodium benzoate (250 mg/kg) was then initiated. On day 222 (19 days after the start of the medication), mercaptopurine hydrate was discontinued. Considering his extremely high ammonia levels, we also began preparations for hemodialysis. However, his ammonia levels quickly dropped and normalized (115 μmol/L at 12 h, 70 μmol/L at 24 h after the initiation of nitrogen scavenger therapy), accompanied by the rapid disappearance of all symptoms. At the onset of the hyperammonemia attack, the nephrotic syndrome persisted; therefore, potassium intake was restricted due to poor potassium excretion caused by tubular damage. The nitrogen scavenger therapy was gradually reduced after referring to the ammonia levels and blood amino acid analysis results and was finally stopped. No neurological sequelae were observed. Plasma amino acid analysis showed high glutamate levels and normal citrulline levels, with urinary organic acid analysis showing the presence of high orotic acid levels. A test for seven different genetic mutations for the UCD showed ornithine transcarbamylase (*OTC*) gene mutation [c. 119G>A, (amino acid change p. Arg40His)]. Thereafter, late-onset OTCD was added to his diagnosis. None of his blood relatives had UCD and he did not present with any symptoms of suspected hyperammonemia. Administration of sodium phenylbutyrate, L-arginine hydrochloride, and carnitine was resumed after the diagnosis of OTCD, and no hyperammonemia was observed. Four cycles of azacytidine (75 mg/m^2 for 3 days) were administered for the bridging therapy before HSCT. The ethical aspects of the use of azacytidine were reviewed and approved by the institutional review board of Saga University Hospital (approval date: September 3, 2018).

Regarding the relapse of JMML, he was pretreated with fludarabine (30 mg/m^2 for 5 days) and melphalan (90 mg/m^2 for 2 days). Subsequently, he underwent unrelated allogeneic bone marrow transplantation on day 405 after the first transplant and on day 165 after the onset of hyperammonemia. Administration of the nitrogen scavenger agents, sodium phenylacetate (250 mg/kg) and L-arginine hydrochloride (125 mg/kg), was resumed in time with the second HSCT (immediately after the genetic diagnosis of OTCD), and HSCT was completed safely without inducing another hyperammonemia attack. We introduced a protein intake limit of 1.5 g per body weight per day. The complete chimera was confirmed on day 29. He shows normal developmental progress, with an intact neurological status, and has not experienced another hyperammonemia attack. Furthermore, he has no abnormal eating habits. His JMML has remained in remission for over 3 years.

DISCUSSION

The present case presents an extremely rare combination of JMML and OTCD. One noteworthy aspect of the present case is the proven involvement of congenital UCD in the pathogenesis of hyperammonemia associated with cancer treatment. The incidence of JMML and OTCD is 1–2/million and 1/80,000, respectively (6, 7, 10), which suggests the extremely low frequency of complicated cases. *OTC* is present at Xp21, whereas *NRAS* is present at 1p13.2. Considering that these two diseases have unrelated genetic mutations, the probability of encountering a case with a combination of JMML and OTCD, as in the present case, was astronomically low. Despite including not only JMML but also hematologic oncology diseases and chemotherapy-related cases in our literature search, we found no reports on hyperammonemia with underlying OTCD.

Chemotherapy-related hyperammonemia was first reported in the 1980s (11, 12), followed by cases occurring after HSCT (13). In most cases, no obvious cause was identified, and the disease was reported as idiopathic hyperammonemia. We have no knowledge regarding any report demonstrating the

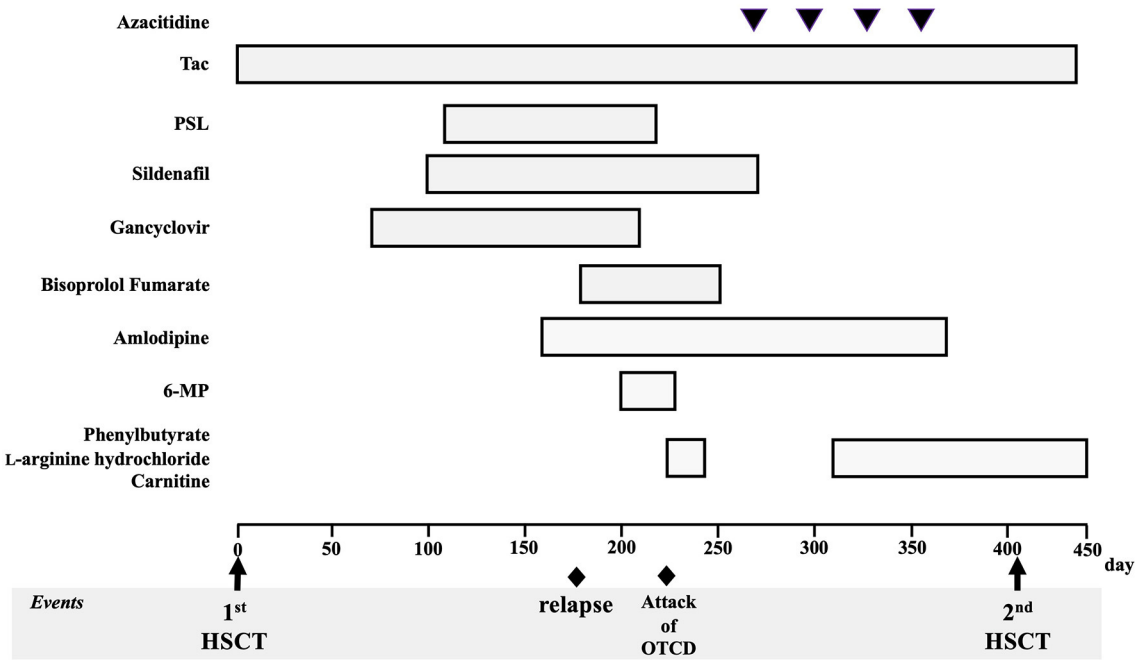

FIGURE 2 | Patient's clinical course and treatment for juvenile myelomonocytic leukemia and complications. Tac, tacrolimus; PSL, prednisolone; 6-MP, 6-mercaptopurine; HSCT, hematopoietic stem cell transplantation; OTCD, ornithine transcarbamylase deficiency.

involvement of an inherited metabolic disease, as observed in the present case. Although there has been a report of those associated with 5-fluorouracil, ammonia is a known metabolite of 5-fluorouracil, with evidence showing a transient and favorable course (14). Only one case has been reported, in which the disease developed during the early steroid treatment phase of acute lymphocytic leukemia (15). No previous reports have suggested an association between hyperammonemia and mercaptopurine, which is the most recently used chemotherapy drug in the present case. There is an exceptional report of a case of acquired carbamoylphosphate synthetase 1 deficiency following transplantation in which the pathogenesis was presumed (16). Chemotherapy-associated idiopathic hyperammonemia may be a multifactorial condition with various onset times, causative agents, and underlying diseases. However, as noted in the present case, UCD should also be considered. Symptoms of hyperammonemia are non-specific and include nausea, vomiting, lack of vigor, seizures, and impaired consciousness, which are relatively frequently encountered during chemotherapy. When abnormal neurological findings are observed, differentiating them from those caused as a result of the disease treatment should be a priority, including direct central damage by anticancer drugs, central nervous system metastasis of malignancy, thrombosis, and hemorrhage, although measuring ammonia levels has also been considered important. In addition, if hyperammonemia is observed, even during chemotherapy, UCD should be considered a potential cause. Therefore, clinicians should have a detailed knowledge of the presenting signs and history of the patient to suspect UCD (17).

OTCD is known to be triggered by starvation, infection, fever, vomiting, excessive protein intake, gastrointestinal bleeding, excess glucocorticoids, and various drugs (18, 19). The severity of male OTCD is correlated with the deleterious mutation that results in structural changes in the OTC protein. In a Japanese nationwide study, c.119G>A of *OTC* mutation was found to be the most common variant and was observed in male patients with late-onset OTCD (20). The timing of the hyperammonemia attacks in the patient was consistent with that observed in the previous reports. In the present case, the patient had good tolerance to various drugs (ganciclovir, tacrolimus, azacytidine, fludarabine, melphalan, and busulfan). It is noteworthy that some of the medications used in the patients included high-dose chemotherapy. High-dose chemotherapy during both transplantations did not trigger hyperammonemic attacks associated with OTCD, suggesting that the intensity of chemotherapy was not necessarily the only factor involved in the development of the disease. We found no reports linking mercaptopurine to hyperammonemic attacks associated with UCDs. However, mercaptopurine most likely triggers hyperammonemic attacks. In our case, this drug was administered for disease control during recurrence; it may have caused tumor collapse, which consequently served as a source of nitrogen. We hypothesized that tumor collapse alone provided a sufficient source of nitrogen to cause the hyperammonemic attack. However, this hypothesis hardly

explains the entire course of our patient's hyperammonemic attack, and no facts can support specific evidence for other causal hypotheses. For example, allopurinol, which indirectly affects the urea cycle, was not administered. The patients' protein intake was approximately 1.5 g/kg/day immediately before the hyperammonemic attack onset. Moreover, the patient did not receive parenteral nutrition. Therefore, mercaptopurine is a highly suspected agent because of its temporal anteroposterior relationship with hyperammonemic attacks. Unfortunately, neither an association nor a pathophysiologically plausible hypothesis could be found in known reports. The onset of OTCD occurred at a time when the child had multiple uncontrollable problems, including post-transplantation relapse and various post-transplantation complications. Davies et al. reported that the post-transplantation onset of idiopathic hyperammonemia occurred at a median onset time of 25 days, with the latest onset occurring at 106 days (13). It can be argued that post-transplantation hyperammonemia, although common during the acute post-transplantation period, is not necessarily unique to the acute period. The timing of the onset of the hyperammonemia attack in the current case was as late as day 223 after the first transplantation. Post-transplantation management requires long-term follow-up, including hyperammonemia. Mitchell et al. reported that postmortem examination of the liver in five patients failed to reveal ultrastructural evidence characteristic of the Reye's syndrome (11). In addition, Frere et al. hypothesized the involvement of gastrointestinal hemorrhage and infection as pathological causes of hyperammonemia after HSCT (21). However, this hypothesis is not strong, and these conditions are not likely to be associated with the induction of seizures in our patient's case. Almost all published studies concluded that post-HSCT hyperammonemia is idiopathic (22). In addition, the present case showed that the second transplantation could be safely managed without acute attacks of OTCD by introducing the nitrogen removal treatment in advance. If hyperammonemia is observed during chemotherapy or after transplantation, we believe that continuing the treatment for hyperammonemia until and after identifying the cause is imperative because many deaths have been reported in several studies regarding unexpected hyperammonemia during chemotherapy (11–13). The clinical outcome of our patient was excellent, and he is currently showing normal developmental range, with an intact neurological status and has not experienced another hyperammonemia attack. His JMML has remained in remission for over 3 years. Kido et al. reported that patients with UCD with a maximum ammonia concentration of >360 μmol/L either died (15%, 11/74) or developed mental retardation (51%, 38/74) (23). Fortunately, our patient is doing well at present and has no neurological sequelae, although his maximum ammonia concentration was above 360 μmol/L.

In conclusion, hyperammonemia should be considered as a differential diagnosis when unexplained loss of consciousness and repeated vomiting occur during hematologic malignancy treatment. Patients should be tested for UCD as a cause of hyperammonemia even under the special circumstances of being on chemotherapy. The present case suggests that even when OTCD diagnosis is confirmed, overcoming highly invasive treatments, such as hematopoietic stem cell transplantation, is possible through adequate preparation and continued administration of hyperammonemic drugs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HE was involved in patient care as well as the drafting, review, and revision of the initial manuscript. TK, KK-I, and KN was involved in the patient's treatment decision as well as the review and revision of the initial manuscript. MN was involved in patient care as well as the review and revision of the initial manuscript. YK was involved in the patient's treatment decision and project administration, as well as the review and revision of the initial manuscript. MM was involved in patient care and project administration, as well as the review and revision of the initial manuscript. All authors have approved the final manuscript submission and have agreed to be accountable for all aspects of the study.

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CASE REPORT
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Abnormal B-Cell Maturation and Increased Transitional B Cells in CBL Syndrome

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CBL syndrome is a Noonan-like RASopathy with heterogeneous clinical phenotype and predisposition to juvenile myelomonocytic leukemia (JMML). Here we describe two patients with identical germline *CBL* mutation and clinical and immune-hematological overlapping features with autoimmune lymphoproliferative syndrome (ALPS) and B-cell expansion with NF-κB and T-cell anergy (BENTA) syndrome. Increased immature/transitional B cells can be depicted in CBL syndrome, ALPS, and BENTA. Nonetheless, our patients here described showed peculiar B-cell phenotype due to increased immature/transitional $CD34^+$ B cells. This feature differentiates CBL syndrome from BENTA, pointing toward an abnormal proliferation of B-cell early precursors.

Keywords: CBL, RASopathies, RALD, BENTA, ALPS, *CARD11*, splenomegaly, lymphocytosis

INTRODUCTION

Germline mutations of the *CBL* gene cause CBL syndrome characterized by variable phenotype involving high frequency of neurologic features, vasculitis, mild Noonan syndrome-like features, and predisposition to juvenile myelomonocytic leukemia (JMML) (1). Indeed, more than 90% of patients with JMML harbor mutations in RAS genes (*NRAS* or *KRAS*) or RAS pathway regulators (*PTPN11*, *NF1*, or *CBL*), which collectively share constitutive activation of RAS/MAPK signaling and therefore also called RASopathies (2). *KRAS* and *NRAS* somatic mutations have been identified in RAS-associated autoimmune leuko-proliferative disorders (RALD), which is associated with overexpansion of lymphocytes with hepatosplenomegaly, lymphadenopathy, and autoimmune phenomena, sharing some clinical and hematopathological features with autoimmune lymphoproliferative syndrome (ALPS) (3).

Although the phenotype of children affected by CBL syndrome has been increasingly delineated over time (4, 5), the immunological features of CBL syndrome have not been extensively described. Here we describe two patients with identical germline *CBL* mutation

[c.1259G > A; (pR420Q)], expanding the immunological phenotypic spectrum of RASopathies and CBL syndrome. Overlapping features with B-cell expansion with NF-κB and T-cell anergy (BENTA) syndrome and RALD are discussed.

An 8-month-old girl with a previous history of severe infections and leukocytosis was referred to our clinic (P1). Family history was unremarkable. At the age of 4 months, she had pneumococcal encephalitis, sepsis, and status epilepticus with subsequent labyrinthitis ossificans. Abdominal ultrasound showed splenomegaly (spleen diameter of 10 cm) and hydronephrosis in the right kidney. In the following months, several urinary tract infections required two admissions and intravenous antibiotics. She presented with speech and psychomotor delay. Right sensorineural hearing loss was demonstrated.

Peripheral blood (PB) tests showed monocytosis (range 1.6–3.69 $\times$ 10^9/l) and thrombocytopenia. B-cell lymphocytosis (range 3.6–5.5 $\times$ 10^9/l) was persistent. B-cell subsets showed a predominant immature/transitional phenotype (**Table 1** and **Figure 1A**). A bone marrow (BM) aspiration showed increased immature B cells ($CD10^{++}CD19^{+}$ $CD20^{+}CD38^{++}$; **Figure 1B**). No dysplastic features were noticed and megakaryocytes were rare. Karyotype was normal. Most of these immature B cells still expressed the CD34 antigen either in PB or BM.

At 3.5 years, JMML was diagnosed based on complete blood count (CBC) with differential, PB, smear findings and detection of CBL variant (6, 7). The CBC showed persistent monocytosis and thrombocytopenia while PB smear myelocytes and metamyelocytes were detected along with anisocytosis in either erythrocytes or platelets, some of whose were giant

TABLE 1 | Patient characteristics at the time of first evaluation.

	Pt1	Age-matched normal values	Pt2	Age-matched normal values
Age, years	0.5		16	
Hemoglobin, g/dl	12.1	11.5–13.5	13.2	12.0–16.0
Mean corpuscolar volume, fl	86.9	75–87	83.5	78–102
White blood cells, 10^9/l	23.61	5.2–11.0	8.48	4.4–8.1
Neutrophils10^9/l	9.43	>1.5	6.57	>1.5
Lymphocytes10^9/l	9.82	3.4–9.0	1.23	1.4–3.3
Monocytes10^9/l	3.69	<1.0	0.58	<1.0
Eosinophils10^9/l	0.51	<0.5	0.07	<0.5
Basophils10^9/l	0.17	<0.1	0.02	<0.1
Platelets10^9/l	111	>140	156	>140
HbF,%	1.1	3–15	0.9	0.1–1.2
IgG, mg/dl	622	351–919	1431	604–1909
IgA, mg/dl	29	10–85	172	61–301
IgM, mg/dl	49	38–204	119	59–297
IgEkU/l	956	<33	84	<33
CD3 + 10^9/l	3.3	1.9–5.9	809	0.72–2.56
CD4 + 10^9/l	2.77	1.4–4.3	546	0.27–1.88
HLADR + , %	3.9	0.8–6.1	2.3	1.6–12.2
Naïve CD45RA + CCR7 + , %	78.8	68.8–91.7	51.3%	20.4–63.6
RTE CD45RA + CCR7 + CD31 + , %	67.7	42.0–79.0	42.3	11.4–48.1
Centr. mem. CD45RA-CCR7 + , %	14.9	5.6–24.2	30.4	18.7–46.2
Eff mem CD45RA- CCR7-, %	5.0	1.5–8.3	17.0	7.1–38.0
Term diff CD45RA + CCR7 -, %	0.9	0.3–5.9	1.2	0.3–9.1
CD8 + 10^9/l	0.27	0.5–1.7	0.16	0.18–0.78
HLADR + , %	3.4	1.6–30.2	2.2	2.7–31.7
Naïve CD45RA + CCR7 + , %	78.6	37.9–90.7	39.7	13.1–66.5
Centr. Mem. CD45RA- CCR7 + , %	3.9	2.0–13.0	5.0	2.6–24.5
Eff Mem CD45RA- CCR7-, %	6.3	1.3–27.2	40.0	10.1–47.4
Term Diff CD45RA + CCR7-, %	11.3	2.1–36.1	15.3	5.2–63.5
CD19 + 10^9/l	4.96	0.61–2.6	0.15	0.09–0.65
RBE CD38 + + CD10 + , %	76.6	16.5–56.5	33.8	2.1–26.1
Naïve IgD + IgM + CD27-, %	17.3	32.2–66.9	50.5	33.7–74.0
CD19 + + CD21low, %	0.7	0.7–6.2	1.2	1.4–13.6
Sw Mem IgD-IgM-CD27 + , %	0.32	0.12–2.3	6.4	2.8–23.4
IgM Mem IgD + IgM + CD27 + , %	1.6	1.6–8.8	7.3	5.1–25.5
Term Diff CD38++ CD27 + CD20-, %	0.42	0.2–8.5	0.7	0.2–8.1
CD3-CD16 + CD56 + 10^9/l	1.18	0.16–0.95	0.18	0.04–0.74

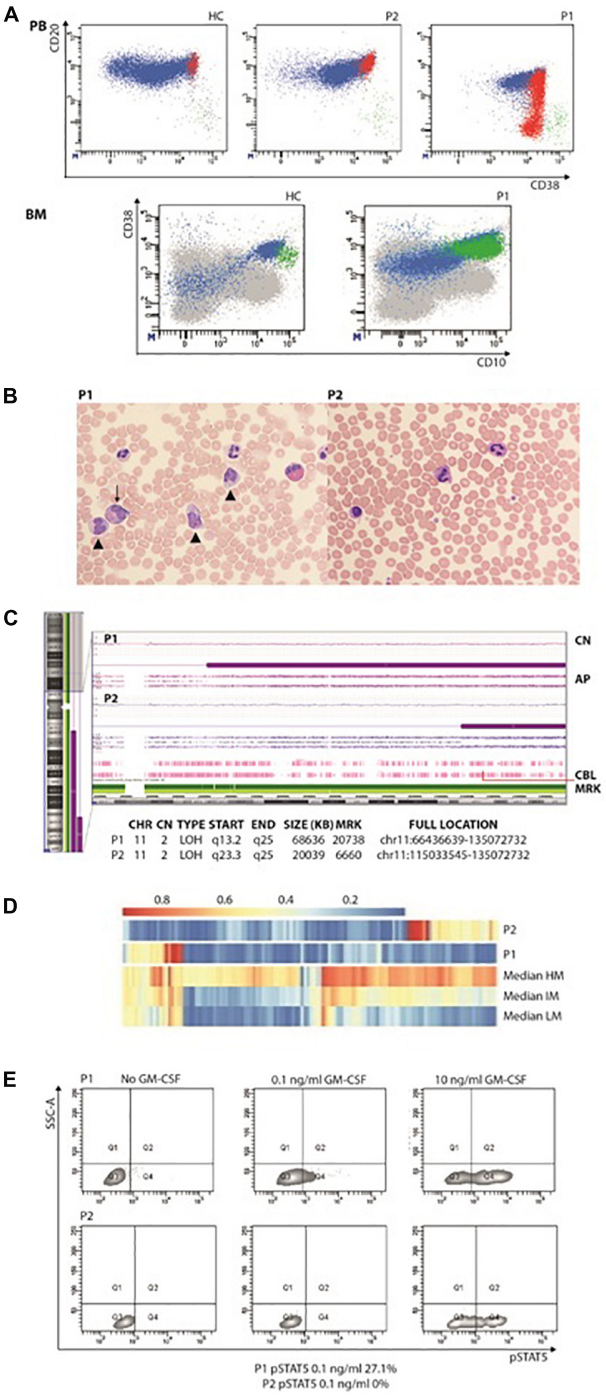

FIGURE 1 | (A) Increased immature/transitional B in the peripheral blood (upper panel; in red are represented $CD10^{+}CD21^{-}CD27^{-}$ transitional or immature B cells, in green $CD10^{-}CD21^{-}CD27^{+}$ terminally differentiated B cells) and bone marrow (lower panel; in green $CD34^{+}CD38^{+}CD10^{+}$). **(B)** Peripheral blood smears showing JMML in P1 (left panel): leukocytosis with monocytosis (triangle) and myeloid precursors (arrows). In the right panel, anisocytosis of platelets without leukocytosis or monocytosis in P2's peripheral blood smear. **(C)** CytoScan HD Array (SNP) analysis: allelic peak (AP) graph showing large copy neutral (CN = 2) loss of heterozygosity (LOH) regions in long arm of chromosome 11, in both patients; common LOH region includes CBL gene at band 11q23.3 (red line). **(D)** DNA methylation patterns in the reported patients showing low-methylation status. **(E)** STAT5 phosphorylation consistent with JMML in P1. AP, allele peaks; CHR, chromosome; CN, copy number state; HC, healthy control; IM, intermediate methylation; HM, high methylation; LM, low methylation; LOH, loss of heterozygosity; MRK, markers/markers number; P1 and P2, patients, START/END, cytoband start/end.

(Continued)

(**Figure 1C**). She has now been followed up for 46 months: over time, thrombocytopenia resolved with the persistence of monocytosis and splenomegaly.

A 16-year-old girl with a previously unremarkable medical history was referred to the Pediatric Hematology outpatient Clinic after splenomegaly was noticed during a routine examination (P2). The patient was born to non-consanguineous parents after an uncomplicated pregnancy. The CBC was normal but the PB smear analysis revealed platelet anisocytosis with giant forms and partially hypergranulated granulocytes were detected (**Figure 1C**). Lymphocyte subsets showed normal values. A BM aspiration showed dysplastic features. Karyotype was normal. The patient is currently 17 years old and in good clinical condition but with splenomegaly.

Both patients showed normal IgG levels. P1 showed decreased IgA and increased IgE, with no sign of atopy. P1's and P2's extensive autoimmunity workup was negative. Both patients had normal level of fetal hemoglobin (HbF).

Severe cytopenia and massive splenomegaly were never observed in both patients; therefore, they were strictly followed up and no further treatment options (6-mercaptopurine and/or low-dose cytarabine or splenectomy) were considered (6).

In both patients' PBMC, the homozygous variant c.1259G > A;[p.Arg420Gln] located in the exon 9 of *CBL* was detected. P1 was investigated by means of target next generation sequencing (NGS) panels, which comprehended primary immunodeficiency defects (including *CARD11*), while trio whole-exome sequencing (WES) was performed in P2. The Sanger sequencing in DNA extracted from hair follicles detected the variant at the heterozygous status, indicating the germline origin in both patients. The single nucleotide polymorphism (SNP) array performed on PBMC suggested that *CBL* mutations were related to the loss of heterozygosity of chromosome 11q that included the *CBL* gene (**Figure 1D**). The DNA methylation profile was predicted to fit the low methylation class (**Figure 1B**). P1 showed p-STAT5 hyper-responsiveness to low doses (0.1 ng/ml) of GM-CSF assayed in $CD33^{+}CD34^{+}$ cells (**Figure 1E**). Due to the identification of CBL mutation, both patients underwent brain magnetic resonance and abdominal Doppler ultrasound without evidence of vasculopathy.

CBL syndrome is a Noonan-like RASopathy with heterogeneous clinical phenotype. Confirming the variant in hair follicles or fibroblasts is crucial in order to prove the germline origin of the genetic lesion and distinguishing Noonan-like syndromes from RALD. To date, more than 50 cases of CBL syndrome have been reported and the majority of them developed JMML (4). JMML is a myeloproliferative/myelodysplastic neoplasm of early childhood characterized by rapidly

progressive disease requiring allogeneic hematopoietic stem cell transplantation as potentially curative treatment in the majority of the patients. Older age, elevated HbF levels, and thrombocytopenia at diagnosis correlate with the poor clinical outcome (6). Other main prognostic factors are represented by genetic subtype defined by RAS pathway mutations and methylation status (8). CBL-mutated JMML can follow an aggressive clinical course or evolve to a spontaneous regression of myeloproliferation with persistence of clonal hematopoiesis (1).

Persistent monocytosis, B-cell lymphocytosis with increased transitional B cells, and splenomegaly have been described in patients affected by RALD (3, 9). BENTA syndrome due to germline gain of function *CARD11* mutations is characterized by congenital lymphoid hyperplasia (particularly splenomegaly) driven by excessive, polyclonal accumulation of B lymphocytes (9). B-cell lymphocytosis was constantly present in P1, resembling the picture already reported in RALD, BENTA, and JMML patients (9). Increased transitional B cells in the PB were detected in both of our patients with T-cell numbers within normal pediatric ranges. Our patients and another patient with CBL reported by Tejwani displayed B subset abnormalities (increased $CD10^+$ immature/transitional B cells) (10).

Moreover, some differences between BENTA, RALD, and CBL syndrome can be drawn. It has been proposed that in BENTA syndrome, B cells may accumulate from increased B-cell output from the BM, as indicated by elevated $CD10^+$ transitional B cells in the PB and consistent with increased immature B cells in the BM (11). Here we have further characterized the B-cell phenotype showing that a consistent number of increased immature/transitional B cells still show the presence of the CD34 antigen (**Figure 1B**), thus pointing toward an abnormal proliferation of B-cell early precursors rather than increased B-cell output from the BM. Recurrent and/or severe infections are common in BENTA syndrome. Although B-cell lymphocytosis has been linked to chronic EBV infection in patients with BENTA (11), P1 did not encounter EBV. CBL patients do not show decreased memory and class-switched B-cell numbers nor hypogammaglobulinemia.

Overall, these cases expand the phenotypic spectrum of CBL syndrome, which overlaps with RALD and BENTA syndrome due to the increased immature/transitional B cells.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Fondazione MBBM, Monza, Italy. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

FS and FV contributed to the conception and design of the study. FS wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Potential role of *MAP2K1* mutation in the trans-differentiation of interdigitating dendritic cell sarcoma: Case report and literature review

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A 5-year-old male child was diagnosed with interdigitating dendritic cell sarcoma (IDCS) during his maintenance therapy for B-cell precursor acute lymphoblastic leukemia (B-ALL). Multiplex lymph node involvements of the neck were found by positron emission tomography CT (PET-CT). Treatments, including surgical and chemotherapy, resulted in complete remission. Four years later, systemic bone infiltration was discovered. Surgical resection of the IV rib and intensive chemotherapy led to a complete morphological remission, and allogeneic bone marrow transplantation was performed. Comprehensive genomic profiling of the formalin fixed the tumor tissue, and the cryopreserved leukemic cells revealed several common alterations and divergent clonal evolution with a novel *MAP2K1* mutation of the IDCS, which is responsible for the trans-differentiation of the common lymphoid-committed tumor progenitor.

KEYWORDS

interdigitating dendritic cell sarcoma, *MAP2K1* mutation, pediatric sarcoma, trans-differentiation, secondary malignant histiocytosis

Introduction

Interdigitating dendritic cell sarcoma (IDCS) is an exceedingly rare dendritic cell neoplasm with even less frequent occurrence among children (1, 2). Based on the recent WHO classification, this entity belongs to the malignant histiocytosis group that also includes histiocytic, Langerhans cell (HS, LCS), and indeterminate cell sarcomas (3). These entities are now considered as "true" hematopoietic tumors arising from bone marrow precursors, together with an L-type histiocytosis, Langerhans cell histiocytosis (LCH), and Erdheim-Chester disease (ECD), while follicular dendritic cell sarcoma

shows a different molecular signature similar to the sarcomas of mesenchymal origin (4, 5). IDCS usually affects lymph nodes, whereas extranodal involvement is infrequent (6, 7). While most of the cases are primary tumors, IDCS may also present as secondary histiocytosis, which term applies to cases where histiocytic/dendritic cell tumors follow or appear simultaneously with another hematopoietic tumor. Secondary histiocytic malignancies have been documented following clonal lymphoid proliferations, such as ALL, CLL, follicular lymphoma, hairy cell leukemia, and diffused large B-cell lymphoma (4, 8, 9). The clonal relationship between the lymphoid and the secondary histiocytic tumors has been proven in several cases with IgH and TCR gene rearrangements, or detection of the same mutation or translocation in both tumors (4, 8, 9). However, detailed analyses of molecular pathways involved in the trans-differentiation process are lacking. Here, we present a secondary pediatric IDCS case featuring an activating *MAP2K1* mutation, possibly driving the trans-differentiation of the common lymphoid-committed tumor progenitor.

Case report

In June 2016, a 4-year-old boy was diagnosed with B-ALL. He presented with petechiae nosebleed, hepatosplenomegaly, WBC of 128 G/L, Hb of 60 g/L, and platelets of 7 G/L. Flow cytometry detected 86% blasts in the bone marrow aspirate with CD34-, CD38-, CD20-, CD10-, and CD58-positive phenotype, and diploid DNA content, and a 9p21 locus deletion found by fluorescence *in situ* hybridization. He was treated according to the intermediate risk arm of the ALL IC-BFM 2009 protocol. He had good prednisone (PRED) response on day 8. On day 15, 0.7% measurable residual disease (MRD) was detected by flow cytometry. On day 33, his bone marrow became MRD-negative. The parenteral chemotherapy was terminated in January 2017. Subsequently, oral maintenance therapy was initiated. Six months later, a hard, painless lymph node of 2 × 1.4 × 1.4 cm size was discovered in the left submandibular region by ultrasound (Figure 1A). Due to progression with structural irregularities, an open biopsy was performed in December 2017.

Histological examination revealed a spindle cell tumor with the following immunophenotype: LCA, CD68, S100, and fascin positivity and negativity for CD1a, CD21, CD23, CD3, CD20, and CD30 (Figures 1B–F), confirming the diagnosis of IDCS. Bone scintigraphy and bone marrow biopsy excluded bone or marrow involvement. The lymph node excision was followed by cervical block dissection, and histology resulted in multiple lymph nodes involvement by IDCS.

The patient received two blocks each of ifosfamide-carboplatin-etoposide (ICE) and adriamycin-bleomycin-vinblastine-dacarbazine (ABVD) chemotherapy, followed by 12 months of oral maintenance with weekly vinblastine (VBL) between June 2018 and June 2019, after which he reached clinical and radiological remission. One year later, multiple enhancing skeletal lesions were identified by the regular follow-up PET-CT (Figures 1G–I). The most intensely affected right IV. rib was resected. IDCS relapse was diagnosed by histology without the presence of residual ALL in the bone marrow. A progressively enlarging cervical lymph node was also resected, although, here, histology indicated only reactive changes. According to the multidisciplinary tumor board decision, from June 2020 to September 2020, he was treated with per os PRED for 5 days/every 4 weeks, combined with weekly VBL. To achieve deep complete remission (CR), his chemotherapy was escalated with two dexamethasone-cisplatin-cytarabine blocks and one more ICE. By November 2020, CR was confirmed by PET-CT. In January 2021, he underwent successful allogenic hematopoietic stem cell transplantation (HSCT). He has remained in CR at the time of this writing (20-month follow-up time), has no identified long-term sequelae, and leads a normal life. He is being followed up by PET-CT and ultrasound scans. The historical timeline of the case is shown in Figure 2.

IgH-gene rearrangement analysis using the Biomed-2 protocol (10) showed identical peaks consistent with the same biallelic or biclonal translocations in the B-ALL and IDCS samples (see Supplementary Figure 1). Comprehensive genomic profiling (CGP) using the Illumina TruSight Oncology500 platform was performed on both IDCS and ALL specimens using a formalin-fixed tumor sample of the lymph node and cryopreserved leukemic cells, respectively (Supplementary material). The CGP found a low mutational burden (4,7 mutation/Mb) in the IDCS specimen. Besides several common alterations mutations of *BTK*, *SOX17*, *DOT1L,* and *ATRX* genes were exclusively identified in the B-ALL sample, while a novel activating mutation of *MAP2K1* in exon2 (c.157_171del) was detected only in the IDCS sample (Figure 2, Supplementary Table 1).

Discussion

Interdigitating dendritic cell sarcoma (IDCS) is a rare aggressive hematopoietic malignancy with poor survival for systemic disease and without standard treatment protocols (6, 11). To date, fewer than 150 cases were reported. Childhood cases represent <10% of them and about 12% of all IDCS cases were presented as secondary neoplasms to other hematopoietic malignancies (6). In 2010, a detailed molecular analysis of LCH and ECD revealed that the proliferation of these tumors is driven by MAP kinase pathway activation (12). Besides the most common mutation of *BRAF*V600E, *MAP2K1* mutation is the second most common alteration found in the *BRAF*

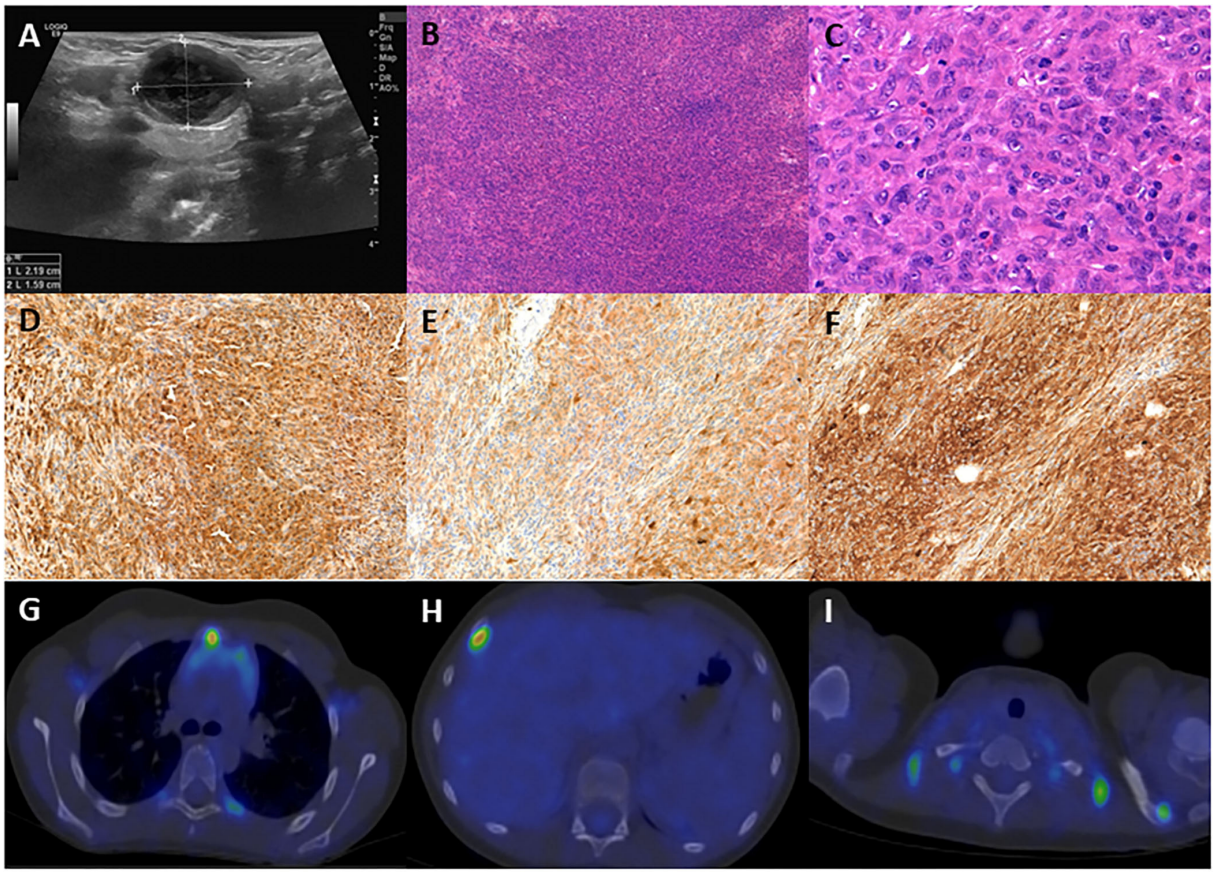

FIGURE 1
(A) Ultrasound picture of the pathologic submandibular lymph node, size: 2.19 × 1.59 cm. **(B)** The excisional biopsy demonstrates diffuse architecture of the lymph node with atypical cell infiltration (100×, H&E). **(C)** The lesion is composed of large, elongated or epithelioid cells with vesicular nuclei, multiplex nucleoli, and eosinophilic cytoplasm (400×, H&E). **(D)** The neoplastic cells are positive for CD68 (100×). **(E)** The neoplastic cells are positive for S100 (100×). **(F)** The neoplastic cells are positive for fascin (100×). **(G)** PET-CT demonstrates infiltration of the sternal manubrium. **(H)** PET-CT demonstrates infiltration of the 4th right rib. **(I)** PET-CT demonstrates infiltration of the acromial end of the left clavicle.

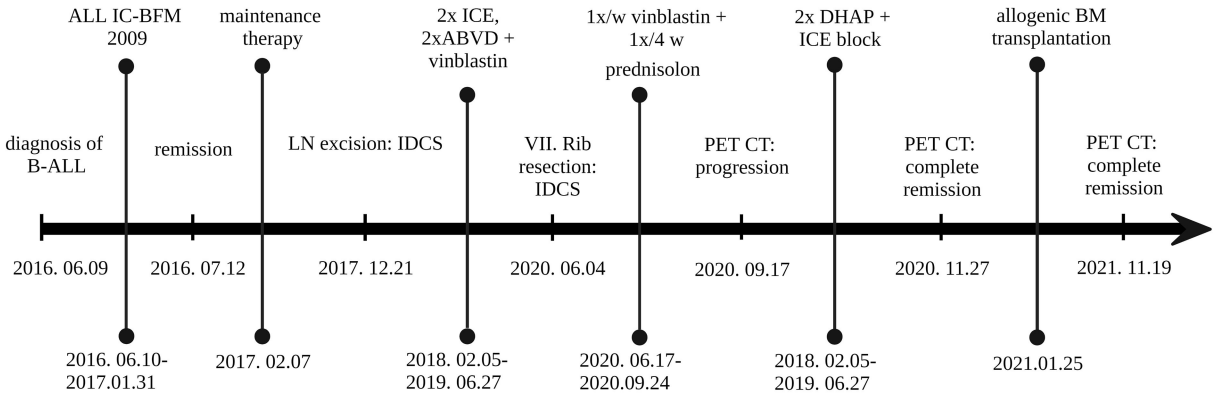

FIGURE 2
Historical timeline of the case. ABVD, doxorubicin (Adriamycin), bleomycin, vinblastine, dacarbazine; B-ALL, B-lymphoblastic leukemia; BM, bone marrow; DHAP, dexamethasone, cytarabine (Ara C), cisplatin; ICE, infusional ifosfamide carboplatin, etoposide; LN, lymph node. Created with BioRender.com.

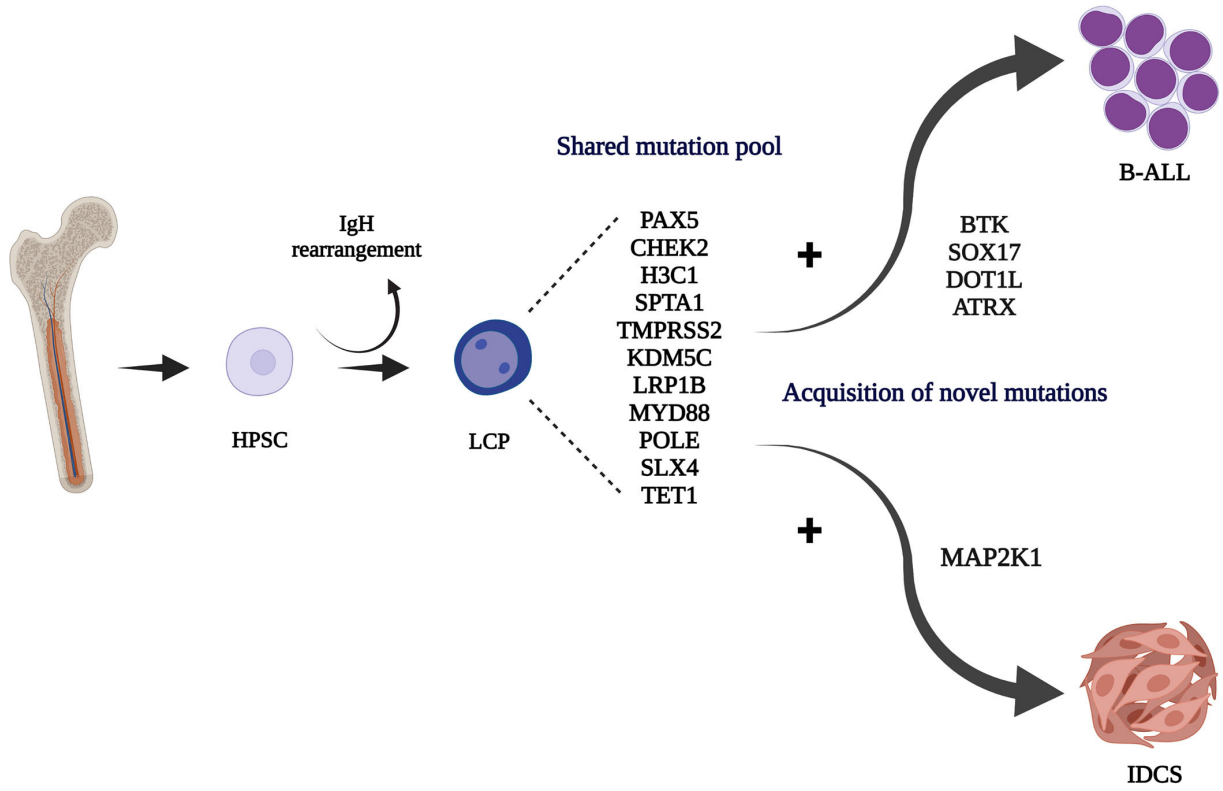

FIGURE 3
Hematopoietic stem/progenitor cell (HPSC) origin of the IgH rearranged common lymphoid committed tumor progenitor (LCP). Acquisition of different driver mutations may contribute to the development of the B-ALL and IDCS clones. Created with BioRender.com.

wild-type cases (13). Involvement of other components of MAP kinase signaling, e.g., *NRAS*, *KRAS*, *ARAF*, and *MAP3K1* mutations, are less frequent and are also described also in the secondary case (14, 15). In patients with systemic histiocytosis, *BRAF* V600E mutation was detected in BM-resident myeloid progenitors, so the cell of origin in these tumors resides in hematopoietic progenitor cells prior to the committed monocyte/macrophage/dendritic cell differentiation (5). In a recent study, beside common *NRAS* and *KRAS* mutations in the hemopoietic malignancies and in the histiocytic tumors, a single case was described with an *MAP2K1* mutation exclusively present in the secondary HS (16). The abundant presence of *IGH*- and *TCR*-rearrangements in sporadic histiocytic tumors indicates that part of these malignancies develop from lymphoid-committed tumor progenitors (17). While recurrent MAPK pathway mutations occur in malignant histiocytosis, e.g., LCS and HS (16, 18, 19), due to its rare occurrence, the molecular background of IDCS is poorly characterized. Recent molecular analyses of few primary and secondary IDCS-cases revealed inactivation of *TP53* (4, 16, 20), while mutation of *SETD2*, *KMT2D*, *ERBB3*, *CDKN2A*, *MET*, and *SF3B1* and amplification of *c-KIT* and *PDGFRα* were reported in single cases (8, 16, 20). MAPK pathway genes shown to carry activating mutations in IDCS are *NRAS* and *BFAF* (4, 21).

Here, we report for the first time a novel *MAP2K1* mutation in a secondary childhood-IDCS case, following B-ALL. Similar activating mutations have been described in 2 LCH cases (c.159_173del) (13, 22). Despite the systemic, relapsing disease, a combined surgical operation and chemotherapy resulted in complete remission, allowing HSCT to offer a final cure (so far, with 20 months of follow-up). This case provides functional evidence linking the cell of origin for this rare malignant dendritic cell tumor to a lymphoid-primed hemopoietic progenitor that could give rise to B-ALL and IDCS by different, specific driver mutations (Figure 3). Involvement of the MAP2K1 pathway in systemic, secondary IDCS tumor development is also confirmed, rationalizing MEK-targeted therapy for refractory cases.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

AJ: collection of the material, data analysis, making the figures, and writing the manuscript. GB: molecular experimental procedures and data analysis. DE and JM: providing clinical data and correction of the manuscript. CB: supervision of experimental procedures, design of pictures, and correction of the manuscript. TG: providing the imaging pictures and correction of the manuscript. ÁS: design of research and writing the manuscript. All authors have contributed to the manuscript, have reviewed and agreed upon the manuscript content.

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Conflict of interest

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Supplementary material

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Case report: A case of acquired von Willebrand syndrome as onset clinical presentation of systemic lupus erythematosus manifested as epistaxis and pulmonary hemorrhage

Songmi Wang[1], Qun Hu[1], Yaxian Chen[1], Xiufen Hu[1], Ning Tang[2], Ai Zhang[1]* and Aiguo Liu[1]*

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Background: Acquired von Willebrand syndrome (AVWS) is a less common bleeding disorder, primarily manifested as mild to moderate mucocutaneous bleeding and laboratory tests are similar to hereditary von Willebrand disease (VWD). AVWS is secondary to other diseases, and systemic lupus erythematosus (SLE) is a relatively rare cause.

Case presentation: We report a case of AVWS as onset clinical presentation of SLE manifested as epistaxis and pulmonary hemorrhage. A 13-year-old male child presented to the hospital with a six-month history of recurrent epistaxis and a one-month history of anemia. Routine blood tests demonstrated severe normocytic anemia and normal platelet count. Von Willebrand test revealed a significantly lower level. High-resolution chest computed tomography (CT) showed patchy ground glass opacities consistent with hemorrhagic changes. After ruling out the family history, the patient was diagnosed with AVWS. Additional tests confirmed positive antinuclear and anti-Sm antibodies. The underlying SLE was diagnosed and treated with methylprednisolone with disease recovery.

Conclusion: We recommend screening for bleeding disorders in patients with recurrent epistaxis. AVWS should be considered when laboratory findings suggest hereditary von Willebrand disease without a personal or familial history of bleeding. In addition, the underlying disease should be explored.

KEYWORDS

epistaxis, pulmonary hemorrhage, pediatrics, acquired von Willebrand syndrome, systemic lupus erythematosus

Introduction

Acquired von Willebrand syndrome (AVWS) is a rare hemorrhagic disease, similar to hereditary von Willebrand disease (VWD) in laboratory tests and clinical manifestations. AVWS primarily occurs in adults without a personal or family history of bleeding diathesis, characterized by mucocutaneous or gastrointestinal bleeding. Laboratory tests reveal prolonged bleeding time and low levels of plasma factor VIII (FVIII) and von Willebrand factor (VWF) measurements (1). Unlike VWD, AVWS is almost associated with an underlying disease. According to a survey of AVWS by the International Society of Thrombosis and Hemostasis (ISTH) in 2000, among the 186 cases, the associated diseases were lymphatic hyperplasia (48%), myeloproliferative diseases (15%), tumors (5%), immunology (2%), cardiovascular (21%) and other diseases (9%) related (2). Systemic lupus erythematosus (SLE) is a rare cause of AVWS. Herein, we report a case of AVWS as onset clinical presentation of SLE manifested as epistaxis and pulmonary hemorrhage.

Case presentation

A 13-year-old male child without significant past medical history or family history presented to the hematology department with a six-month history of recurrent epistaxis and a one-month history of anemia. Besides, he was prone to be bruised recently. The boy was found to be obese and pallor through physical examination. Old ecchymosis on the extremities and waist, blood scab in the bilateral nasal vestibule but without significant bleeding in the oropharynx could be noticed (**Figure 1**). Additionally, he had tachycardia (115 beats/min) and hepatosplenomegaly.

On initial laboratory tests, the patient had severe normocytic anemia with hemoglobin (Hb) of 58 g/L (MCV: 87.4fL, MCH: 27.0pg, MCHC: 309g/L, reticulocytes: 2.87%) and normal platelet count. Coagulation function detection showed normal activated partial thromboplastin time (APTT), prothrombin time, and fibrinogen but slightly elevated D-dimer and fibrin degradation products (FDP). As the child had a severe bleeding diathesis, further screening of platelet function tests was performed and turned out to be normal. Subsequent Von Willebrand test revealed a significantly lower level: von Willebrand factor antigen (VWF: Ag) was 19.9%, and von Willebrand factor ristocetin cofactor (VWF: RCo) was 22.7%

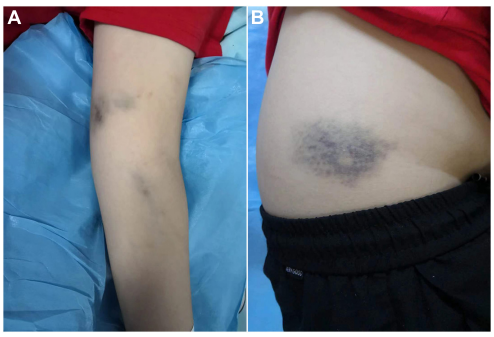

FIGURE 1
(A) Ecchymosis on the upper extremity. **(B)** Subcutaneous hemorrhage on the waist.

(**Table 1**). His parents' studies were negative for hereditary VWD. Therefore, AVWS was diagnosed.

The underlying cause required further investigation. Considering that the patient had normocytic anemia with elevated reticulocytes, the Coombs test was carried out with a

TABLE 1 Laboratory data of the patient during his hospitalization.

	Initial	Post-steroids	Final	Reference values
WBC	5.03	8.95	5.74	3.50–9.5 × 10^9/L
Hb	58	81	121	130.0–175.0g/L
PLT	246	383	178	125.0–135.0 × 10^9/L
Reticulocytes	2.87%	/	1.87%	0.5–1.5%
Coombs	++	/	negative	negative
ESR	/	16	72	0–15mm/H
ALT	19	45	24	≤41U/L
AST	34	45	24	≤40U/L
albumin	34	31.2	16.6	32–45g/L
Cr	67	50	42	59–104ummol/L
IgG	29.2	/	4.3	7.0–15.6 g/L
C3	0.18	/	0.37	0.65–1.39 g/L
C4	0.02	/	0.13	0.16–0.38 g/L
ANA	1:3200	/	1:3200	negative
anti-Sm	>8.0	/	>8.0	<1.0
anti-dsDNA	negative	/	1:100	negative
LA	negative	/	negative	negative
PT	14.2	14.5	12.5	12.0–14.5s
APTT	44.9	33.5	40.4	32.0–45.0s
Fibrinogen	3.9	2.56	5.95	2.0–4.0 g/L
VWF:Ag	19.9	56.5	119.2	50–200%
VWF:Rco	22.7	75.4	102.5	50–200%
Hematuria	negative	negative	+	negative
Proteinuria	negative	negative	+++	negative

ALT, alanine aminotransferase; ANA, antinuclear antibodies; anti-dsDNA, anti-double stranded DNA antibodies; anti-Sm, anti-Smith antibody; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; C3, complement component 3; C4, complement component 4; Cr, creatinine; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; IgG, immunoglobulin G; LA, lupus anticoagulant; PLT, platelet count; PT, prothrombin time; WBC, white blood cell count; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor. +, positive; ++, moderate positive; +++, strong positive.

Abbreviations: APTT, activated partial thromboplastin time; AVWS, acquired von Willebrand syndrome; CT, computed tomography; FDP, fibrin degradation products; FVIII, factor VIII; MRI, magnetic resonance imaging; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; SLICC, systemic lupus international collaborating clinics; VWF, von Willebrand factor; VWD, von Willebrand disease; VWF: Ag, von Willebrand factor antigen; VWF: RCo, von Willebrand factor ristocetin cofactor.

positive result. Additional tests confirmed positive antinuclear antibody (1:3200) and anti-Sm antibody. Lupus anticoagulants were negative. The immunologic testing revealed a higher immunoglobulin G level and significantly lower complement levels (C3: 0.18g/L, C4: 0.02g/L). According to Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE (3), the underlying SLE was diagnosed. To further clarify the disease activity index, the following examinations were performed. Although the child had no symptoms such as fever, cough, dyspnea, chest pain, or hemoptysis, lung CT showed diffuse ground-glass nodules consistent with hemorrhagic changes. With the exclusion of infectious lesions, pulmonary manifestations in Systemic Lupus Erythematosus were considered (**Figures 2A,B**). Urinalysis and color Doppler ultrasound of the urinary system, and magnetic resonance imaging (MRI) of the head, showed no abnormalities. Echocardiography revealed left atrial enlargement and a small amount of pericardial effusion. Mild fatty liver and splenomegaly were found in color ultrasound of the liver and spleen. According to the above, this patient's systemic lupus erythematosus disease activity index (SLEDAI) (4) was 4 points.

We did not choose desmopressin, VWF-containing concentrates, or intravenous immunoglobulin for hemorrhage control, considering that the child had no acute bleeding or adequate funding. The patient received pulse therapy with methylprednisolone (500 mg/d) for five days, and VWF activity (VWF: Ag 56.5%, VWF: RCo: 75.4%) increased to the normal level. Then the patient had no further active bleeding. In a follow-up to the nine months since he was diagnosed, no further epistaxis or ecchymosis occurred during this period, which was confirmed by his subsequent normal coagulation function and VWF levels. His chest CT also recovered (**Figures 2C,D**). Unfortunately, the serologic tests suggested higher levels of antinuclear antibodies (1:3200) and strongly positive anti-double-stranded DNA antibodies (1:100). What was worse, his kidneys were affected, shown as nephrotic syndrome included heavy proteinuria (urine protein: + + +, 24-h urinary protein quantification: 5.09g), hypoalbuminemia (16.6g/L), peripheral edema, and hyperlipidemia (total cholesterol 11.97mmol/L, triglycerides 2.30 mmol/L). SLEDAI's score was as high as 22 points. Renal biopsy showed lupus nephritis (WHO type V).

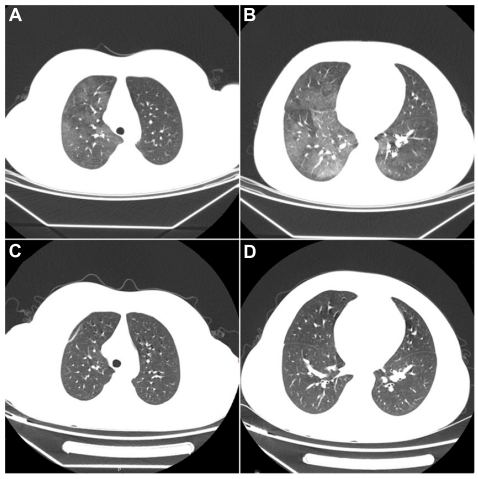

FIGURE 2
(A,B) Diffuse ground-glass opacities consistent with hemorrhagic changes in both lungs. **(C,D)** After treatment, repeated CT shows complete disappearance of the abnormal lesions.

Discussion

Acquired von Willebrand syndrome (AVWS) is a less common bleeding disorder, often unrecognized or mistaken for von Willebrand disease. In 1968 Dr. Simone et al. (5) first reported an adolescent with SLE and AVWS. Subsequently, AVWS has regained attention due to its relevance in cardiovascular diseases, including congenital heart defects, aortic stenosis, and using left ventricular assist devices (6). Additionally, AVWS is associated with many other underlying disorders, such as solid tumors, hematological cancers, and autoimmune diseases. More than 20 patients with AVWS and SLE have been reported (7–10).

Most cases with AVWS present mild to moderate mucosal bleeding without a personal or familial history of bleeding. Laboratory tests are similar to hereditary VWD, characterized by prolonged bleeding time and decreased von Willebrand factor levels (e.g., decreased VWF: Ag, decreased VWF: RCo, and/or abnormal high-molecular-weight multimers). FVIII levels may be decreased, and APTT may be prolonged. In this case, the detection of VWF was not performed until six months after the onset of symptoms, despite skin ecchymosis and epistaxis at presentation to the hospital.

Nosebleeds are common in children, with 75% of children experiencing at least 1 episode of epistaxis (11). The nose is well vascularized. The Kiesselbach plexus, located in the anterior nasal cavity, is the main source of epistaxis in children (12). The nasal mucosa provides little anatomical support and protection to the underlying blood vessels, resulting in nasal vascular congestion or any factor that dries or irritates the nasal mucosa can increase the likelihood of epistaxis. Common causes of epistaxis include dry nasal mucosa, sinusitis, trauma, foreign bodies, etc. However, repeated epistaxis should consider the possibility of systemic diseases such as bleeding disorders and tumors (13, 14). Unfortunately, the boy's parents and the attending physician were likely falsely reassured by his young age and a large amount of exercise.

The pathophysiology of AVWS is not fully understood. Most AVWS patients have normal or even increased VWF synthesis and release, but the removal of VWF from plasma is significantly increased, except for patients with hypothyroidism.

Current studies suggest the pathogenic mechanisms underlying AVWS may be related to the following mechanisms: autoantibodies to VWF or FVIII inhibit VWF function and increase the clearance of the FVIII-VWF complex (15); adsorption of VWF by tumor cells, or activated platelets increase the clearance of the FVIII-VWF complex (16); cell-mediated or drug-induced increase proteolysis of VWF multimers (1, 6). Currently, the first mechanism involving circulating antibodies to VWF is considered the primary mechanism of AVWS in SLE patients.

For AVWS, the effective treatment of hemorrhage occurs when the underlying disease and the autoantibodies are controlled (17, 18). Current treatments are mainly for primary diseases and pathogenic mechanisms, including desmopressin, VWF-containing concentrates, intravenous immunoglobulin, recombinant factor VIIa, antifibrinolytics, and plasmapheresis (19–24). For AVWS and SLE, steroids and cyclophosphamide are effective in controlling hemorrhage (25–28). Recent studies have reported that rituximab is used to treat AVWS in adolescent SLE (29–31), and bleeding in both patients has been effectively controlled. In this case, the activity of VWF and FVIII returned to normal levels after methylprednisolone pulse therapy. The primary disease control of the child in a later stage is not ideal, but there is no bleeding diathesis. It suggests that the remission of the primary disease is not always consistent with AVWS (17).

Therefore, it is necessary to perform the relevant examinations for unexplained bleeding. Patients with clinical manifestations and laboratory findings similar to hereditary VWD should be considered AVWS after excluding family history. It is essential to explore the underlying disorders. The key to controlling bleeding is the diagnosis and treatment of the primary disease.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

SW contributed to the acquisition, analysis of data, and writing the manuscript. NT, YC, AZ, XH, and QH performed the diagnostics and treatment of the patient. AL, QH, and AZ conceived the idea and reviewed the manuscript. All authors have substantively revised the work and approved the final submitted version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case report: Leukemia cutis as the first manifestation of chronic neutrophilic leukemia in a 6-year-old girl

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Chronic neutrophilic leukemia (CNL) is a rare *BCR-ABL* negative myeloproliferative neoplasm that usually affects older adults with a poor prognosis. Leukemia cutis is an extramedullary manifestation of leukemia and may be misdiagnosed by dermatologists. Here, we describe a case of CNL in a 6-year-old Chinese girl with leukemia cutis as the first manifestation. Her skin rashes failed to attract the attention of dermatologists in early stages. The diagnosis was confirmed by peripheral smear, bone marrow studies, genomic analysis and skin biopsy.

KEYWORDS

leukemia cutis, chronic neutrophilic leukemia, child, skin, *CSF3R*

Introduction

Chronic neutrophilic leukemia (CNL) is a rare *BCR-ABL* negative myeloproliferative neoplasm that is characterized by neutrophilia, splenomegaly, and poor prognosis (1). CNL usually affects older adults with a median survival of approximately 2 years and only approximately 200 cases of CNL have been reported worldwide (2). Activating mutations in the colony-stimulating factor 3 receptor (*CSF3R*) gene have been identified in most cases of CNL (3). The most common mutation is T618I and has been introduced to the diagnostic criteria for CNL in the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia (4). Leukemia cutis is an extramedullary manifestation of leukemia and is associated with a worse prognosis (5). Here, we described a case of CNL in a 6-year-old Chinese girl with leukemia cutis as the first manifestation.

Case description

A 6-year-old girl presented with a 10-month history of repeated cutaneous plaques and abscesses. She was initially diagnosed with furuncle, and the abscess improved after rupture. Similar rashes occurred repeatedly every 1–2 months and were resolved

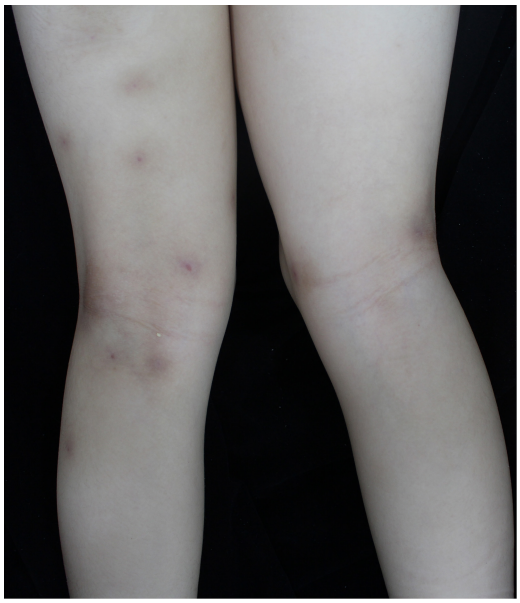

FIGURE 1
Multiple dull red plaques were observed on the lower limbs.

within 1 week. The plaques and abscesses reoccurred in the limbs 4 weeks prior. The abscesses disappeared after rupture 3 weeks prior, but arthralgia occurred in the limb joints 2 weeks prior. On physical examination, multiple dull red plaques were observed on the lower limbs (**Figure 1**) and splenomegaly with spleen 3 cm below the left costal margin was also noted. The complete blood count was described as follows: white blood cell count: 105.32 × 10^9/L, neutrophils: 87.3%, lymphocytes: 9.1%, monocytes: 0.9%, eosinophils: 0.3%, basophils: 2.4%, red blood cells: 3.01 × 10^{12}/L, hemoglobin: 99 g/L, and platelet count: 190 × 10^9/L. The peripheral smear revealed a predominance of mature neutrophils with rare blasts (1%). Bone marrow studies revealed hypercellular marrow with granulocytic hyperplasia (**Figure 2**), negativity for *BCR/ABL* and a + 8 karyotype. Genomic analysis revealed a *CSF3R* T618I somatic mutation and a *CBL* c.1096-1_1097delGGA frameshift somatic mutation (**Figure 3**). Next-generation sequencing from her blood cells did not detect any other associated gene mutations. Skin biopsy revealed a few blasts infiltrated in the dermis without dermal edema (**Figure 4**). The blasts expressed CD33, CD68, CD117, Ki67 (5%+), and MPO but not CD2, CD3, CD30, CD56, or TdT. Then, a diagnosis of CNL with leukemia cutis was made. The patient was initially treated with hydroxyurea for 1 month. After the *CSF3R* somatic mutation was confirmed, the therapy was changed to ruxolitinib subsequently. The white blood cell count decreased to 13.16 × 10^9/L, the skin lesions improved, and the spleen had shrunk to 1.6 cm below the left costal after 3 months treatment. She received the allogeneic hematopoietic stem cell transplantation subsequently. At present, she is in stable condition and under follow-up.

Discussion

In CNL patients with skin lesions, sweet syndrome and leukemia cutis are difficult to differentiate because of clinical and histological similarities (6). Differentiating these conditions is important because sweet syndrome is not associated with the prognosis while leukemia cutis is a predictor of a worse prognosis (7). Sweet syndrome has an abrupt and painful onset of skin lesions which are associated with fever and improved with steroids (8). Histological examination shows a predominantly mature neutrophilic infiltrate with prominent papillary dermal edema (8). In contrast, leukemia cutis features the gradual onset of non-tender skin lesions that do not improve

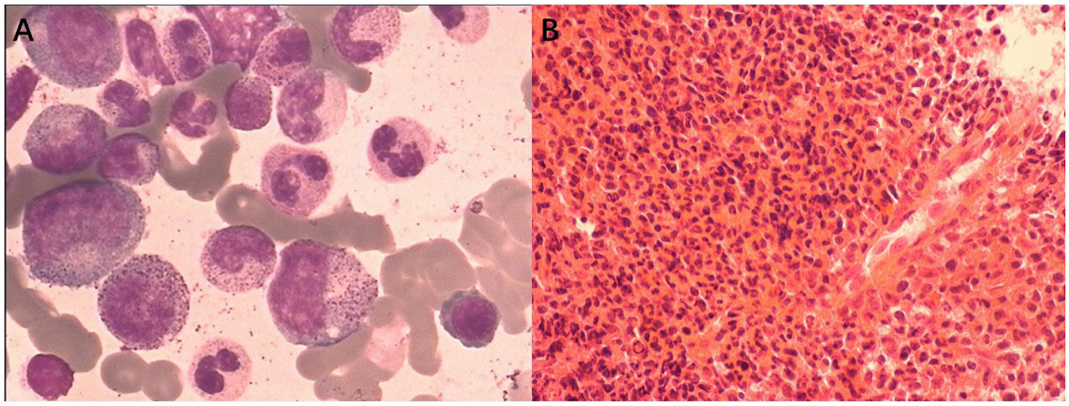

FIGURE 2
Bone marrow studies revealed hypercellular marrow with granulocytic hyperplasia. **(A)** Bone marrow smear (Wright's stain; original magnification, ×400). **(B)** Bone marrow biospy (Hematoxylin-eosin stain; original magnification, ×100).

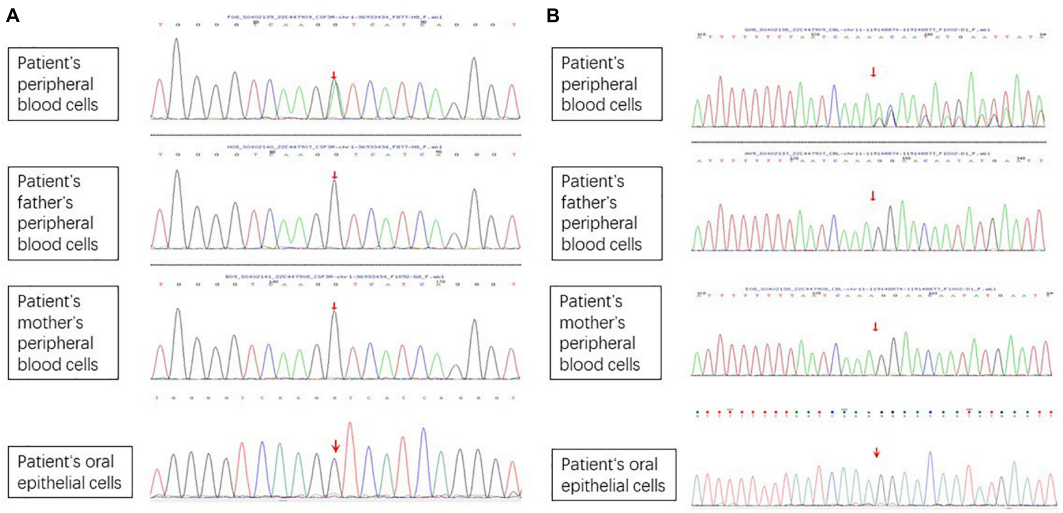

FIGURE 3
Genomic analysis revealed a CSF3R T618I somatic mutation and a CBL c.1096-1_1097delGGA frameshift somatic mutation. **(A)** CSF3R T618I somatic mutation. **(B)** CBL c.1096-1_1097delGGA frameshift somatic mutation.

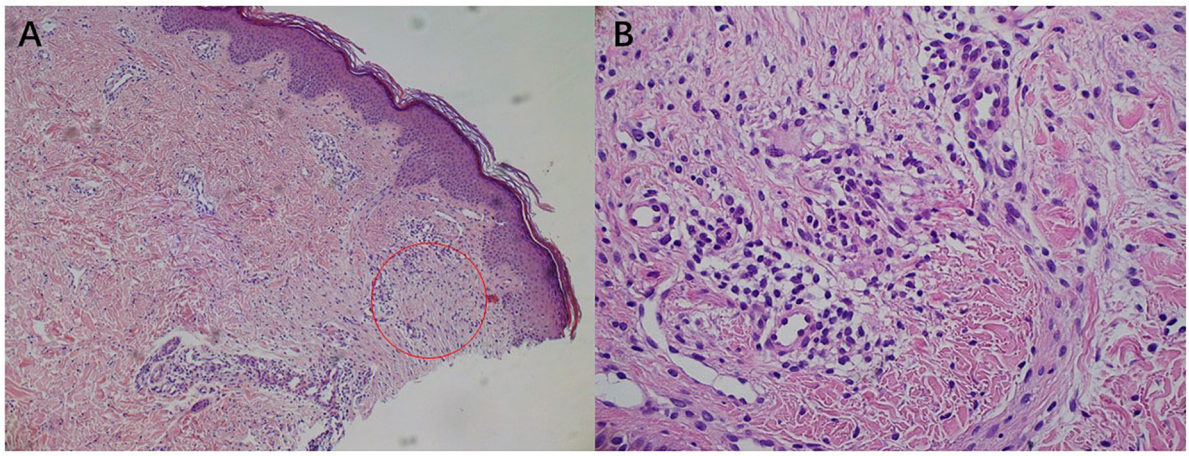

FIGURE 4
Skin biopsy showed immature blasts infiltrate. **(A)** Hematoxylin-eosin stain; original magnification, ×100. **(B)** Hematoxylin-eosin stain; original magnification, ×400.

with steroids (8). Histological examination shows immature blasts infiltrate without significant dermal edema (8).

Chronic neutrophilic leukemia is a very rare disease and CNL with leukemia cutis is even rarer. To our knowledge, this case is the fifth case of CNL with leukemia cutis and the second case of CNL with leukemia cutis as the first manifestation. The first case of CNL with leukemia cutis as the first manifestation was presented with multiple erythematous to violaceous papules and excoriations on both lower extremities in a 70-year-old man (3). The lesions were improved with hydroxyurea and cefazolin therapy but not respond to oral steroid and empiric antibiotics therapy (3).

Chronic neutrophilic leukemia patients with leukemia cutis are considered with an aggressive course and short survival (8). In fact, the first CNL patient with leukemia cutis died 5 months after eruption (9). However, the differences between CNL patients with leukemia cutis and patients without leukemia cutis are still not well-known because of the low prevalence. Further studies should be conducted to illustrate the differences in epidemiology, treatment protocols, and survival.

The skin lesions of this patient were gradual onset plaques with abscesses and histological examination showed immature blasts infiltrate without dermal edema. These findings confirmed the diagnosis of leukemia cutis. Regretfully, skin biopsy was

conducted after abscesses rupture. Therefore, only a few immature blasts were observed under histological examination. The special feature of this patient was that the leukemia cutis was the first manifestation, and it appeared far earlier than the appearance of arthralgia. She visited the dermatology department in the early stages. However, her repeated skin rashes failed to attract the attention of dermatologists. Therefore, it is necessary to remind dermatologists that chronic recurrent plaques and abscesses may be manifestations of leukemia cutis.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

RW and JY were responsible for the diagnosis and treatment of the patient. YZ and JY prepared the manuscript. RW and ZX participated in the revision of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Pediatric immune myelofibrosis (PedIMF) as a novel and distinct clinical pathological entity

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Myelofibrosis is a rare myeloproliferative disorder. The detailed descriptions of myelofibrosis in children and adolescents is limited to a few case series and case reports describing fewer than 100 patients, thus suggesting the extreme rarity of this condition prior to adulthood. Though pediatric patients rarely present the typical features and outcomes usually observed in older people, pediatric myelofibrosis is not considered an independent entity. Here we aim to describe patients with pediatric myelofibrosis, showing different clinical and pathological features when compared to the World Health Organization 2016 Primary Myelofibrosis classification. We retrospectively collected and analyzed 14 consecutive pediatric myelofibrosis diagnosed in our Pediatric hematology outpatient clinic over a six-year period. According to clinical data and bone marrow biopsy findings, patients were classified into three subgroups: adult-like myelofibrosis, pediatric immune myelofibrosis, idiopathic myelofibrosis. Pediatric Immune Myelofibrosis was the predominant subgroup in our cohort (7/14). Pediatric Immune Myelofibrosis is characterized by peculiar bone marrow features (i.e., T lymphocyte infiltration) and a milder course compared to the other patients Pediatric Immune Myelofibrosis is a novel and distinct pathological entity. We suggest to carefully consider Pediatric Immune Myelofibrosis in case of bone marrow biopsies showing myelofibrosis that do not fulfill WHO criteria.

KEYWORDS

myelofibrosis, bone marrow, reticulin fibrosis, autoimmune myelofibrosis, inborn errors of immunity, pediatric immune myelofibrosis

Introduction

Myelofibrosis (MF) is a clonal myeloproliferative condition, characterized by dysregulated proliferation of megakaryocytes, myeloid and erythroid cells, extramedullary hematopoiesis associated with reactive bone marrow (BM) fibrosis, osteosclerosis, angiogenesis, extramedullary hematopoiesis and abnormal cytokine

expression (1). MF can occur *de novo* (primary MF or PMF) or secondary to the development of marrow fibrosis after polycythemia vera or essential thrombocythemia (secondary MF) or in association with chronic myeloid leukemia (2, 3). In 2003, Pullarkat et al. described "primary autoimmune myelofibrosis" (AIMF) as MF occurring in patients presenting autoimmune (AI) biological signs in the absence of a well-defined autoimmune disease (AID). Conversely, the term "secondary AIMF" was used for MF occurring with a well-defined AID (4).

MF and particularly PMF in children are exceedingly rare. The detailed descriptions of children and adolescents with PMF and AIMF are limited to a few case series and case reports describing fewer than 100 patients (5–8), thus suggesting the extreme rarity of this condition. According to WHO 2016, the diagnosis of PMF (pre-PMF and overt PMF) requires all three major criteria and at least one minor criterion. The Major criteria include: Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often decreased erythropoiesis (pre-PMF)/Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis (grade 2 or 3) (overt PMF); Not meeting WHO criteria for BCR-ABL1 + CML, PV, ET, MDS, or other myeloid neoplasm; Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker or absence of minor reactive BM reticulin fibrosis. The Minor criteria include one or more of these findings, confirmed in two consecutive determinations: Anemia not attributed to a comorbid condition; Leukocytosis $\geq 11 \times 10^9$/L; palpable splenomegaly; LDH level above the upper limit of the institutional reference range; Leukoerythroblastosis (only for overt PMF) (9). Despite pediatric patients rarely show the typical features and outcomes observed in PMF, the World Health Organization (WHO) 2016 classification has not defined Pediatric Myelofibrosis (PedMF) as an independent entity (9). These lack of data on PedMF implies the absence of a consensus regarding proper treatment and management of PedMF.

We describe the characteristics and outcomes of fourteen consecutive patients with PedMF treated in our center from 2015 to 2021. Clinical, histological, and genetic features of PedMF are quite different compared with those reported in adult MF. Our results show that MF may occur in children with conditions such as autoimmune disorders, inborn error of immunity (IEI, not yet described as associated with MF), and other heterogeneous molecular causes, which we collectively define as Pediatric Immune Myelofibrosis (PedIMF). Such a pattern was the most frequent form of PedMF and laboratory, clinical, histological data and need for treatment reveal that PedIMF may be considered a distinct clinical pathological entity.

Methods

We performed a retrospective analysis (**Figure 1**) of patients' examinations performed in our Pediatric Hematology Unit between January 2015 and December 2021 ($n = 32{,}146$) screening our institutional database. Patients' examinations were included if one or more of the following inclusion criteria were satisfied: (i) patients referred for isolated or multiple cytopenia (anemia, thrombocytopenia, neutropenia, leukopenia), thrombocytosis, erythrocytosis, leukocytosis, eosinophilia, splenomegaly; (ii) BM biopsy performed between January 1st, 2015–December 31st, 2021; (ii) presence of reticulin fibrosis above or equal to grade 1 [sec. WHO 2016 (9)]. The exclusion criteria were as follows: (i) patients who had hematopoietic stem cell transplantation (HSCT), or chemotherapy (ii) patients diagnosed with hemoglobinopathies, inherited blood cell disorder (i.e., Fanconi anemia, Blackfan Diamond Anemia, hereditary spherocytosis) or neoplastic disorders.

We collected the reports from screened patients' examinations ($n = 7{,}499$). We selected patients who performed BM biopsy ($n = 65$) and they were included if BM presented with reticulin fibrosis above or equal to grade 1 ($n = 28$). Nine records were excluded as diagnosed with other conditions (i.e., SAMD9L syndrome, $n = 1$; BMF due to *DNAJC21* variants, $n = 2$; Hoyeraal-Hreidarsson syndrome, $n = 1$; hemochromatosis, $n = 1$; essential thrombocythemia $n = 2$, aplastic anemia, $n = 2$). Finally, a cohort composed of 14 patients performing 18 BM threphine biopsies (four individuals performed an additional BM biopsy each) was identified. Medical records and complete blood counts, peripheral blood (PB) smears, hepatic and renal function tests, BM aspirate and biopsy findings, cytogenetics and molecular profile were collected and analyzed. Infections, AI disorders, endocrine work-up and malignancies were also evaluated. BM biopsies were reviewed for histopathologic features of MF including cellularity, vascularity, osteosclerosis megakaryocytic hyperplasia and clustering, dysmegakaryopoiesis, myeloid hyperplasia, myeloid to erythroid ratio, morphologic dysplasia and immunohistochemical staining. Immunophenotyping (CD34, CD117, CD20, CD3) was performed in case of immune disorder or autoantibodies positivity. Fibrosis was graded as normal, mild, moderate, or severe (MF-0 to MF-3), according to the consensus criteria of Thiele et al. (10). BM biopsies were examined in parallel by two hemopathologists (F.P., V.L.) and minor discordances during the evaluation were solved by consensus. Molecular analyses were performed in eleven out of fourteen patients, following clinical decisions on a case-by-case basis (**Table 1**). Targeting next generation sequencing panels are detailed in **Supplementary Tables S1, S2**. For continuous variables, mean and standard deviation (SD) or quartiles (Q1, median and Q3), as appropriate, were calculated, while qualitative variables were reported as count

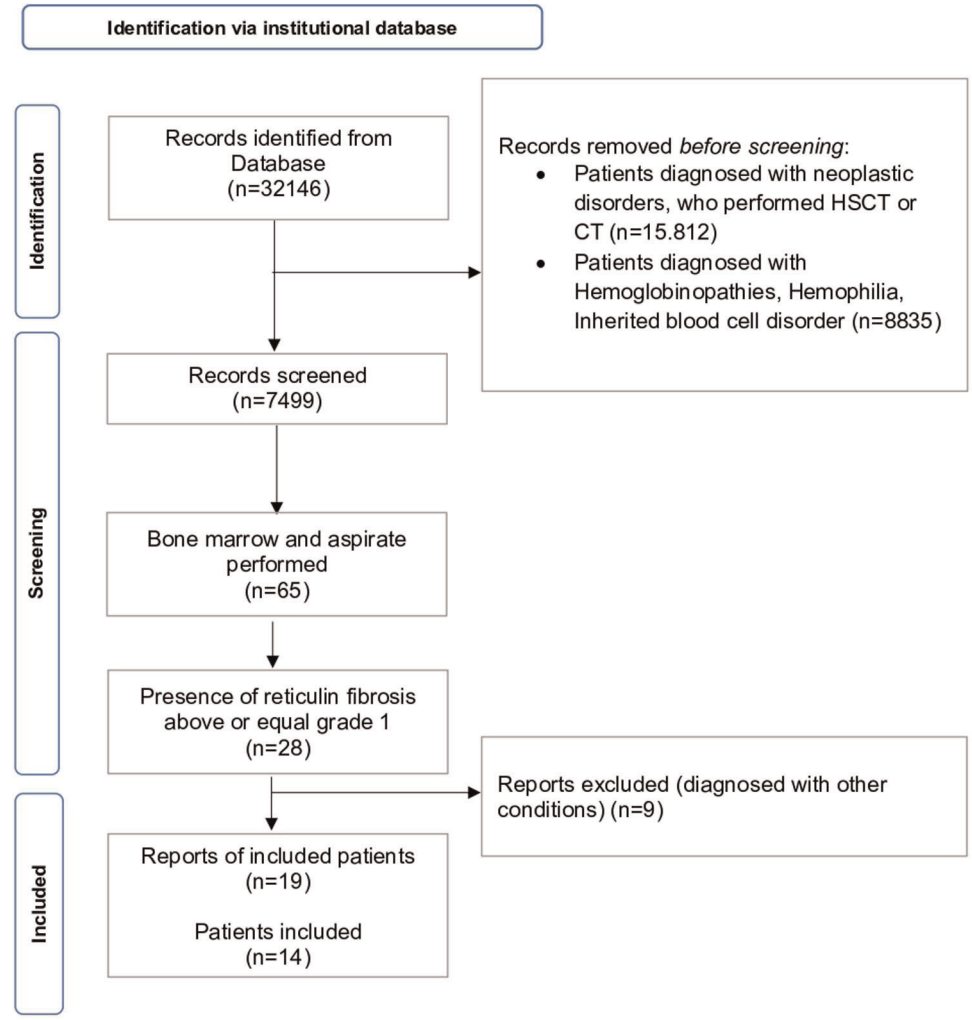

FIGURE 1
PRISMA flowchart of patients' retrieval, inclusion and exclusion of records. CT = chemotherapy, HSCT = hematopoietic stem cell transplantation.

TABLE 1 Molecular analysis performed in the included patients (n = 14).

Analysis	Number of investigated patients (%)
JAK2 V617F	11/14 (78%)
MPL exon 10	3/14 (21%)
CALR exon 9	3/14 (21%)
FHL associated genes	1/14 (7.1%)
GATA2	1/14 (7.1%)
NGS panel A	7/14 (50%)
NGS panel B	5/14 (35.7%)
Whole exome sequencing	1/14 (7.1%)

and frequency. Chi-square and ANOVA tests were used to compare dichotomous and continuous variables, respectively. Included patients were classified as follows:

- "Adult-like PMF" in case of patients who either met all the WHO 2016 criteria or presented the typical features of PMF (with or without the presence of molecular lesions) (9);
- "Pediatric Immune MF" (PedIMF) in case of patients presenting with AI predisposing syndromes (11), autoantibodies without defined disorders (4) or known IEI (12);
- idiopathic MF in those not fulfilling the above defined criteria.

Results

Clinical and laboratory profile

Clinical and laboratory data of the 14 included patients are summarized in **Tables 2**, **3**. Seven patients being female and

TABLE 2 Clinical and presenting features of enrolled patients ($n = 14$). ASA, acetylsalicylic acid; FH Syndrome, familial hemophagocytic lymphohistiocytosis (FHL) syndrome.

	Age (years) or number of patients (%)
Age	
Median	13 years, IQR: 8 years
Gender	
Male	$n = 7$ (50%)
Organomegaly	$n = 5$ (35.7%)
Blood count	
Anemia	$n = 7$ (50%)
Thrombocytopenia	$n = 3$ (21.4%)
Thrombocytosis	$n = 2$ (14.3%)
Pancytopenia	$n = 1$ (7.1%)
Eosinophilia	$n = 1$ (7.1%)
Genetic abnormalities	
Trisomy 21	$n = 2$ (14.3%)
JAK2V617 mutation	$n = 1$ (7.1%)
CALR	$n = 1$ (7.1%)
GATA2	$n = 1$ (7.1%)
STXBP2	$n = 1$ (7.1%)
Reticulin Fibrosis	
MF-1	$n = 6$ (42.9%)
MF-2	$n = 6$ (42.9%)
MF-3	$n = 2$ (14.3%)
Bone marrow	
Hypercellular	$n = 5$ (35.7%)
Normocellular	$n = 6$ (42.9%)
Hypocellular	$n = 3$ (21.4%)
Megakaryocytic hyperplasia	$n = 7$ (50%)
Megakaryocytic clustering	$n = 5$ (35.7%)
Dysmegakaryopoiesis	$n = 11$ (78.6%)
Intrasinusoidal hematopoiesis	$n = 4$ (28.6%)
Lymphocytic infiltration	$n = 5$ (35.7%)
Osteosclerosis	$n = 0$
Pediatric Immune Myelofibrosis	
Sjogren syndrome	$n = 1$ (7.1%)
GATA2 deficiency	$n = 1$ (7.1%)
FHL syndrome	$n = 1$ (7.1%)
Down syndrome	$n = 2$ (14.3%)
Autoimmune neutropenia	$n = 1$ (7.1%)
L-HES	$n = 1$ (7.1%)
Treatment	
Steroid course	$n = 2$ (14.3%)
ASA treatment	$n = 2$ (14.3%)
RBC and PLT transfusions	$n = 6$ (42.9%)
Chemotherapy	$n = 1$ (7.1%)
HSCT	$n = 2$ (14.3%)
Splenectomy	$n = 1$ (7.1%)
Outcome and follow-up	
Median time of follow-up	5.4 years, IQR: 4.25 years
Alive	$n = 14$ (100%)

IQR, interquartile range; FHL, familial haemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; MF-1, reticulin fibrosis grade 1; MF-2, reticulin fibrosis grade 2; MF-3, reticulin fibrosis grade 3; RBC, red blood cells; PLT, platelets.

seven male, respectively. The median age at the time of diagnosis was 13 years old (range 1–18 years). There was no family history of hematological disorders except for one patient whose father and sibling were affected by anemia and thrombocytopenia, even though those relatives were not available for further investigations. Two patients presented with a family history of solid malignancies (melanoma, thymoma, testicular cancer) or AI thyroiditis each. Systemic symptoms were rarely reported. At the time of the first clinical evaluation, four patients had splenomegaly. None had a history of severe, recurring or acute infections. No patient had jaundice, lymphadenopathy, gout, bone pain, night sweats, bleeding, congestive cardiac failure, renal dysfunction, thrombosis, portal hypertension or hepatic failure. The most common cytopenia was anemia ($n = 7$, mean hemoglobin 10.9 g/dl; range, 3.7–16.3 g/dl) followed by thrombocytopenia ($n = 3$, mean platelet counts 53×10^9/L, range $7–70 \times 10^9$/L). One patient had pancytopenia. Two patients had thrombocytosis (mean platelet count 1019×10^9/L, range $146–1892 \times 10^9$/L). The mean leucocyte count was 10.300/mm^3. One individual had eosinophilia.

Classification of pediatric mf

Four patients were classified as having "adult-like PMF" (Pt1–Pt4). Two patients met all the WHO criteria (Pt2 and Pt3), including molecular lesions (*JAK2*, $n = 1$; *CALR*, $n = 1$). A further *ASXL1* variant was detected in one patient (Pt3) who already carried one *CALR* somatic variant. Two patients presented the typical features of PMF without the presence of molecular lesions (Table 3). Increased LDH and leukoerythroblastosis were consistently documented (Pt1–Pt4). Splenomegaly was detected in two patients (Pt2, Pt4). Three out of four patients in this group required treatment. Two children (Pt1, Pt3) needed haematopoietic stem cell transplantation (HSCT) after fully myeloablative conditioning from mismatched unrelated donors. One patient (Pt2), with thrombocytosis and *JAK2* variant, is currently treated with low dose acetylsalicylic acid (ASA).

Three patients (Pt5-Pt7) were classified as idiopathic MF. None of them displayed molecular markers or increased LDH. Two patients (Pt5, Pt7) presented with pancytopenia.

TABLE 3 Comparison between WHO 2016 criteria and pediatric MF criteria of the 14 included patients.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Major criteria WHO 2016														
Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often decreased erythropoiesis (prePMF)/ Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis (grade 2 or 3) (overt PMF)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Not meeting WHO criteria for BCR-ABL1 + CML, PV, ET, MDS, or other myeloid neoplasm	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker or absence of minor reactive BM reticulin fibrosis	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No	No
Minor criteria WHO 2016														
Palpable spleen	No	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	No	No	No
Anemia	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No	No
LDH Increased	Yes	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes	–	–	Yes
Leukocytosis $>11 \times 10^9$/L	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Leukoerythroblastosis	No	No	Yes	Yes	–	–	–	–	–	No	No	–	–	–
Pediatric Myelofibrosis														
Proposed PedMF Classification	Adult-like	Adult-like	Adult-like	Adult-like	Idiopathic MF	Idiopathic MF	Idiopathic MF	Immune MF	Immune MF	Immune MF	Immune MF	Immune MF	Immune MF	Immune MF
PedMF criteria	Fulfilling WHO criteria except for molecular markers, BM findings consistent with adult PMF	Fulfilling WHO criteria	Fulfilling WHO criteria	Fulfilling WHO criteria except for molecular markers, BM findings consistent with adult PMF	None	None	None	Autoantibodies positivities (ANA, Coombs); FHL	GATA2 deficiency	Autoantibodies positivities (Lac +), Down's Syndrome	ITP, Down's Syndrome	AIN	Sjiogren's Syndrome	L-HES

AIN, autoimmune neutropenia; BM, bone marrow; CML, chronic myeloid leukemia; ET, essential thrombocythemia; FHL, familial haemophagocytic lymphohistiocytosis; ITP, immune thrombocytopenia; L-HES, lymphocyte-variant hypereosinophilic syndrome; MDS, myelodysplastic syndromes; PMF, primary myelofibrosis; PV, polycitemia vera; WHO, world health organization.

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Splenectomy was performed in one patient (Pt5) due to massive splenomegaly causing pancytopenia and requiring red blood cell transfusions.

Seven individuals (Pt8–14) were classified as having PedIMF (Table 3). Two patients had IEI, of whom one (Pt9) was diagnosed with GATA2 deficiency when she was 20 years old, while the second one had a concurrent diagnosis of PedIMF and FHL (13). Down's syndrome was diagnosed in two patients (Pt10 and Pt11) in the first year of life. They subsequently developed PedIMF when they were 3 and 14 years old, respectively. One reumatological condition (Siogren Syndrome) was diagnosed in Pt13 at 16 years old. 3 years later, PedIMF was demonstrated. Two patients (pt 12 and Pt 14) presented with hematological conditions (Autoimmune neutropenia and L-HES). BM biopsies showed PedIMF when they were 13 and 10 years old, respectively. Four patients (Pt8–Pt12) had autoantibodies positivity prior to PedIMF. Ana, LAC and Coombs positivity were detected at 7 (Pt8), 2 (Pt10), 5(Pt11) years of age, respectively. PB counts of patients with PedIMF presented with variable features, such as anemia ($n = 4$), thrombocytopenia ($n = 2$), neutropenia ($n = 1$), eosinophilia ($n = 1$) and pancytopenia ($n = 1$). None of them exhibited leukoerythroblastosis. Four children (Pt8, Pt10, Pt11, Pt14) displayed increased LDH. Splenomegaly was detected in two patients (Pt8, Pt10).

Pathology data

BM findings are outlined in Tables 3, 4. The main and most frequent histological differences between Adult-like and PedIMF are reported in Figure 2. Adult-like MF showed an increased

TABLE 4 Summary of immunePedMF, idiopathic pedMF, adult-like PMF.

	Immune PedMF ($n = 7$)	Idiopathic PedMF ($n = 3$)	Adult-like PMF ($n = 4$)	*p*-value
Age (y)				
MEDIAN (years/IQR)	10 years 7 years	4 years 11 years	9 years 8.5 years	
Gender				
Male	$n = 3$ 42.8%	$n = 3$ 100%	$n = 2$ 50%	
Female	$n = 4$ 57.2%	–	$n = 2$ 50%	
Organomegaly	$n = 2$ 28.6%	$n = 1$ 25%	$n = 2$ 50%	
Blood count				
Anemia	$n = 3$ 42.8%	$n = 2$ 50%	$n = 2$ 50%	
Thrombocytopenia	$n = 2$ 28.6%	$n = 1$ 25%	–	
Thrombocytosis	–	–	$n = 2$ 50%	
Cytopenia	$n = 1$ 14.3%	$n = 1$ 25%	–	
Eosinophilia	$n = 1$ 14.3%	–	–	
Molecular marker or genetic lesion				
Normal (*n*, %)	$n = 3$ 42.8%	$n = 3$ 100%	$n = 2$ 50%	
Trisomy 21	$n = 2$ 28.6%	–	–	
JAK2V617 mutation	–	–	$n = 1$ 25%	
CALR	–	–	$n = 1$ 25%	
GATA2	$n = 1$ 14.3%	–	–	
STXBP2	$n = 1$ 14.3%	–	–	
Cellularity and stromal changes				
Cellularity, mean percentage and range	69 % 20%–90%	70 % 50%–80%	90 % 80%–90%	
Hypercellularity per age (*n*, %)	2 , 29%	1 , 33%	3 , 75%	0.304
Myelofibrosis [MF] (mean ± SD)	1.29 ± 0.76	2 ± 1	2 ± 0.82	**0.029**
Increased vascularity and/or intrasinusoidal hematopoiesis (*n*, %)	0	1 , 33%	3 , 75%	**<0.01**
Megakaryocytes				
Hyperplasia (*n*, %)	2 , 29%	1 , 33%	3 , 75%	0.304
Clustering (*n*, %)	1 , 14%	0	3 , 75%	**0.021**
Dysmegakaryopoiesis (*n*, %)	4 , 57%	3 , 100%	4 , 100%	0.314
Myeloid hyperplasia (*n*, %)	1 , 14%	0	3 , 75%	**0.021**
Erythroid hyperplasia (*n*, %)	3 , 43%	3 , 100%	1 , 25%	0.267
T lymphocytes, CD3+ (mean percentage ± SD and range)	13.6% ± 7.5 10%–30%	11.7% ± 7.6 5%–20%	8.3%| ± 7.9 5%–20%	**0.029**

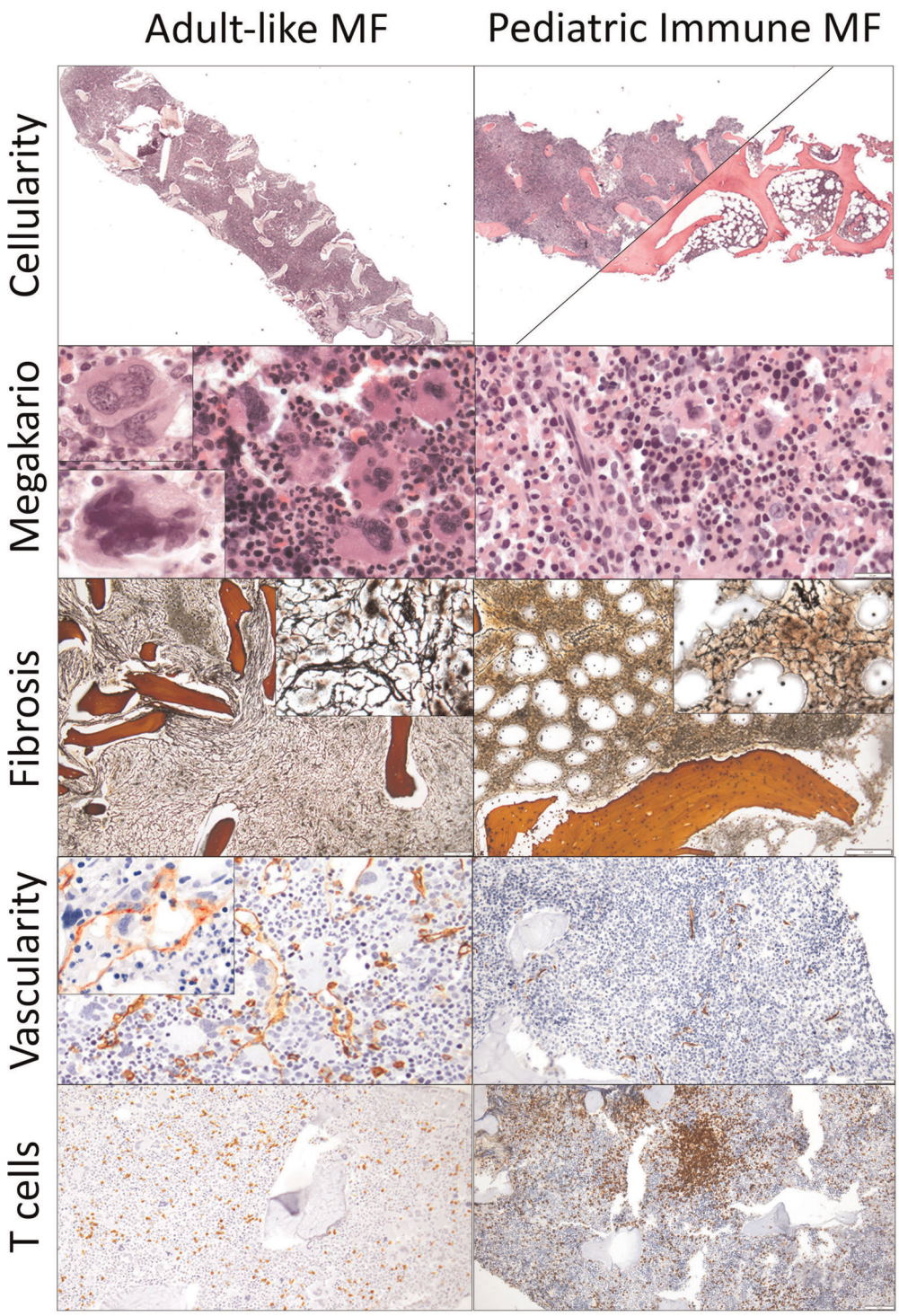

FIGURE 2
Comparison of the histological features of adult-like MF and PedIMF in this cohort adult-like MF demonstrated higher cellularity (90% in average, **A**, H&E, ×20), frequent clustering of megakaryocytes (**B**, H&E, ×400), with either hyperplastic/bulbous (upper left inset) or dysmorphic (bottom left inset) forms. Moreover, the increase in reticulin was generally high (2 ± 0.82, **C**, H&E, ×100) with frequent thickening and intersection of fibers (upper right inset), with prominent microvascular proliferation (CD34 IHC, **D**, ×200) and occasional intrasinusoidal hematopoiesis (upper left inset) but relatively preserved percentage of infiltrating T lymphocytes (8.3% ± 7.9, CD3 IHC, **E**, ×100). On the other hand, PedIMFshowed a variable cellularity, ranging from 90% to 20% (**F**, H&E, ×20), with almost normal or rarely hyperplastic megakaryocytes (**G**, H&E, ×500), relatively lower increase in reticulin (1.29 ± 0.76, **H**, H&E, ×100) with only occasional intersection of fibers (upper right inset). Microvasculature was often preserved (CD34 IHC, **I**, ×200) and a significant increase in the T lymphocytes infiltration, often arranged in aggregates, was noted in the majority of cases (13.6% ± 7.5, CD3 IHC, **J**, ×100).

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cellularity per age ($n = 3$, 75%), with an average of 90% as compared to the adipose component, moderate increase in marrow fibrosis (MF 2 ± 0.82) and prominent vascularity frequently associated with intrasinusoidal hematopoiesis ($n = 3$, 75%) (Table 4, Cellularity and stromal changes section). These cases showed significant hyperplastic changes of megakaryocytes ($n = 3$, 75%), with frequent formation of dense clusters alternating bulbous and dysmorphic elements (Table 4, Megakaryocytes section). On the other hand, PedIMF demonstrated variable cellularity per age, ranging from 20 to 90%, with lower marrow fibrosis on average (MF 1.29 ± 0.76, $p = 0.029$) and an almost preserved vascularity (Table 4, Cellularity and stromal changes section). In idiopathic MF and PedIMF megakaryocytes were normal or only slightly increased, rarely in clusters and with less dysmorphic features (Table 4, Cellularity and stromal changes section). Myeloid hyperplasia was more prominent in the adult-like MF ($n = 3$, 75%) whereas erythroid hyperplasia was more frequent in the idiopathic forms ($n = 3$, 100%) (Table 4, Myeloid and Erythroid hyperplasia section). Finally, no significant increase of the B-lymphocytes was observed, whereas PedIMF cases showed a relatively higher infiltration by T lymphocytes, with PedIMF and idiopathic MF forms showing relatively higher ($13.6\% \pm 7.5$ and $11.7\% \pm 7.6$, respectively) percentage of CD3 + cells in the BM biopsy as compared to adult-like MF ($8.3\% \pm 7.9$, $p = 0.029$), often with the formation of interstitial aggregates (Table 4, T lymphocytes section).

Treatment and follow up

Treatment and follow up data are summarized in Table 2.Median time of follow up at the last visit was 5.4 years (Q25 = 0 years; Q75 = 4.25 years). All patients are alive and well at the time of the last follow up. Ten out of 14 patients are currently followed up in our Pediatric Hematology Outpatients Clinic. Among individuals diagnosed with PedIMF, two required supportive care. Two patients required steroid treatment. Pt14 required a short steroid course, while Pt8 required long-term steroid treatment due to autoimmune hemolytic anemia. Chemotherapy was administered in Pt11 for subsequent diagnosis of acute myeloid leukemia. Three patients (Pt4, Pt11, Pt14) repeated BM aspirate and biopsy and results are shown in Table 5. Cellularity decreased in two out of three patients and reticulin fibrosis in all of them.

TABLE 5 Summary of repeated bone marrow aspirates and biopsies.

Patient	4	8	11	11	14
Molecular marker or genetic lesion	–	STXBP2	Tr.21	Tr.21	–
Cellularity %	50%	90%	80%	90%	50%
Reticulin Fibrosis (MF)	1	2	1	0	1
Increased vascularity	Present	Absent	Absent	Absent	Absent
Intrasinusoidal hematopoiesis	Present	Absent	Absent	Absent	Absent
Osteosclerosis	Absent	Absent	Absent	Absent	Absent
Megakaryocytic hyperplasia	Absent	Absent	Present	Absent	Absent
Megakaryocytic clustering	Absent	Absent	Present	Absent	Absent
Dysmegakaryopoiesis	Present	Present	Absent	Absent	Absent
Palpable spleen	Absent	Absent	Absent	Absent	Absent
Anemia	Absent	Absent	Present	Absent	Absent
Leukoerythroblastosis	–	–	Absent	–	–
LDH Increased	–	Absent	–	Absent	–
Leukocytosis $>11 \times 10^9$/L	Absent	Absent	Absent	Absent	Absent

Discussion

Myelofibrosis is characterized by PB cytopenia, leukoerythroblastosis, ineffective hematopoiesis, proliferation of dysfunctional megakaryocytes with reticulin and/or collagen fibrosis in the BM, and dysregulated cytokine production as well as extramedullary hematopoiesis and hepatosplenomegaly (14). A detailed description of Pediatric MF is limited to a few case series focused on PMF (5–7) and AIMF (4, 8). Authors agree that clinical, histological, and molecular features of Pediatric MF differ from adult PMF, but given the rarity of the disease, precise data are lacking. Pediatric MF has a heterogeneous phenotype with variable outcomes ranging from spontaneous resolution to a rapidly progressive, sometimes fatal, disease curable only by HSCT. Indeed, different authors have proposed to consider Pediatic MF as a distinct entity when compared to PMF (14).

We believe that the heterogeneous phenotype of Pediatric MF calls for a classification that includes further subtypes among these patients. Our results seem to identify three defined subgroups of children with MF. The first group includes patients with features that are typically detected in adult patients with PMF (Adult-like PedMF). Laboratory values, commonly seen in adult PMF patients—including increased LDH, bilirubin, and leukoerythroblastosis—all together suggest high marrow cell turnover and are well documented in these children (15). Driver mutations in genes such as *JAK2*, *CALR*, or *MPL* mutations can be detected in the "Adult-like PMF" subgroup. BM hypercellularity, dysmegakaryopoiesis, megakaryocytic hyperplasia, megakaryocytic clustering and myeloid hyperplasia and increased vascularity are common findings. When compared with adult PMF patients reported in the literature, "Adult-like PMF" seems to differ with respect to clinical symptoms. In fact, constitutional symptoms (like fatigue, weight loss, night sweats) are often lacking, despite splenomegaly is present (14). Finally, the need for and the type of treatment administered

(i.e., HSCT and ASA) are shared between children with adult-like PMF and PMF.

In the second and most represented group of patients, MF occurs in the context of IEI, known rheumatological or hematological conditions or autoantibodies positivity. We define these children as having PedIMF. In our case series along with predominantly conditions that present with autoantibody and B cell alterations, we expand the concept of immune PedIMF to predominantly T-cell (L-HES) and innate immune defects (GATA2, Familial haemophagocytic lymphohistiocytosis Syndrome). Apart from splenomegaly and increased LDH, which are common findings in PedIMF and are shared with the adult-like cohort, patients with PedIMF present some peculiar features, namely PB results, BM findings and lack of need of treatment. Although PB counts in PedIMF are typically variable, they essentially lack leukoerythroblastosis. These features differentiate PedIMF from Adult-like MF and Idiopathic MF; the latter was mainly characterized by cytopenia. Differences between adult-like MF and PedIMF are evident on the BM examination as well. Indeed, this novel entity is characterized by a variable marrow cellularity, with hypo and hypercellular cases, a relatively lower grade of increase in reticulin, a substantially preserved interstitial vascularity and less prominent myeloid hyperplasia. Another histological hallmark of PedIMF is represented by the prevalence of T lymphocyte infiltration, often organized in central aggregates, stressing the possible link with the underlying autoimmune/IEI disorder (16). In the PedIMF group, in fact a milder course has been observed compared to other groups of our cohort, as already previously reported. Although our series suggests that the majority of MF cases have a benign course with spontaneous resolution, one patient (Pt10) affected by Down Syndrome after being diagnosed with PedIMF, subsequently developed malignant evolution (Acute myeloblastic leukemia). Although the relationship between trisomy 21 and MF has been already described (17, 18) this association can be only speculated due to lack of data.

Idiopathic MF exhibits extreme variability compared to the other groups. Distinctive features are represented by normal level of LDH, absence of megakaryocytic clustering or increased vascularity in BM specimens. Reticulin fibrosis was markedly different, ranging from grade 1 to grade 3. The disease course in the idiopathic PedMF is unpredictable, yet we have to consider the small number of patients.

Given the striking difference in disease course and prognosis between PedIMF and the other entities it is imperative to have reliable diagnostic criteria. Thus, after having re-examined the clinical, laboratory, genetic and morphologic findings of our cohort we highlighted the role of BM T lymphocyte infiltration. Clinicians should be aware that previously unreported AI, immunological, rheumatological conditions may be associated with PedIMF and therefore consider it in the follow-up of these patients. On the other hand, in case of BM infiltrate by T-lymphocytes, AI and IEI workup should be performed in those patients who have no defined diagnosis.

There are some limitations to this study. The number of patients is small, yet the rarity of this disorder has to be taken into account. Genetic testing could have been not uniformly undertaken in all patients due to the retrospective pattern of this analysis. However, our pediatric series characterized three different subgroups of patients, underlining as predominant the immune histopathological and genetic features in a detailed manner, documenting that MF in children is a different entity when compared with adults.

Conclusions

MF in children have histopathological and genetic features different from adults and it mainly occurs in the context of IEI, known rheumatological or hematological conditions or autoantibodies positivity, collectively we refer to this novel entity as PedIMF. These findings underscore the importance of appropriate diagnostic work-up and treatment of the underlying disorder causing MF.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Fondazione MBBM, Monza. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

FG and FS contributed to conception and design of the study and wrote the first draft of the manuscript. FP and VL examined BM biopsies. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.1031687/full#supplementary-material.

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Splenic rupture and fungal endocarditis in a pediatric patient with invasive fusariosis after allogeneic hematopoietic stem cell transplantation for aplastic anemia: A case report

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Background: Invasive mold infections are a well-known and life-threatening condition after allogeneic hematopoietic stem cell transplantation (HSCT). While *Aspergillus* species are recognized as predominant pathogens, *Fusarium* species should also be considered due to their broad environmental distribution and the expected poor outcome of invasive fusariosis. Particularly, splenic rupture as a complication of disseminated disease has not been reported yet.
Case presentation: Two weeks after allogeneic HSCT for severe aplastic anemia, a 16-year-old boy presented with painful, erythematous skin nodules affecting the entire integument. As disseminated mycosis was considered, treatment with liposomal amphotericin B and voriconazole (VCZ) was initiated. Invasive fusariosis was diagnosed after histological and previously unpublished polymerase chain reaction-based examination of skin biopsies. Microbiological tests revealed *Fusarium solani* species. Despite stable neutrophil engraftment and uninterrupted treatment with VCZ, he developed mold disease-associated splenic rupture with hypovolemic shock and fungal endocarditis. The latter induced a cardiac thrombus and subsequent embolic cerebral infarctions with unilateral hemiparesis. Following cardiac surgery, the patient did not regain consciousness because of diffuse cerebral ischemia, and he died on day +92 after HSCT.
Conclusion: Invasive fusariosis in immunocompromised patients is a life-threatening condition. Despite antimycotic treatment adapted to antifungal susceptibility testing, the patient reported here developed uncommon manifestations such as splenic rupture and fungal endocarditis.

KEYWORDS

invasive mold infection, fusarium, endocarditis, splenic rupture, hematopoietic stem cell transplantation, case report

Introduction

Bacterial, viral, and fungal infections are frequently observed in children and adolescents after allogeneic hematopoietic stem cell transplantation (HSCT). Invasive fungal disease is a life-threatening condition (1). Of particular concern are disseminated infections by mold species which may require the combination of drug treatment and surgical measures to achieve optimal outcomes (2). Major risk factors for infections by mold species are extended periods of neutropenia, the systemic use of steroids, and the presence of chronic graft-versus-host disease (GvHD) (1). While *Aspergillus* species represent the most predominant pathogens in these patients, *Fusarium* species also need to be considered due to their broad, environmental distribution (3). Here, we report a fatal course of *Fusarium solani* infection in an adolescent after allogeneic HSCT for aplastic anemia. A rupture of the spleen was observed in our patient as a hitherto unique manifestation of disseminated fusariosis. However, the most critical and finally fatal event in the post-transplant course was fungal endocarditis with subsequent embolization into the brain from a cardiac thrombus.

Case presentation

A 16-year-old male patient was diagnosed with very severe aplastic anemia (VSAA) after a brief history of petechiae and pale skin. The results of the standard diagnostic procedures for acquired bone marrow failure had ruled out acute leukemia, Fanconi anemia, dyskeratosis congenita (DC), paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS), and a primary immunodeficiency (PID) syndrome. The patient's family history was uneventful. Allogeneic HSCT from an HLA-identical brother was postponed for several months as the family hoped that identifying a causal agent and its treatment could reverse the effects on the bone marrow. Supportive care during these few months included frequent, mostly weekly transfusions with platelets and packed red cells and antimicrobial prophylaxis with topical amphotericin B, oral fluconazole, and cotrimoxazole. The patient's neutrophil count was permanently below 500 µl. Consent for HSCT was obtained after 6 months of neutropenia when the patient had developed a threatening soft tissue infection by *Streptococcus anginosus* in his left hand, requiring surgical treatment.

The patient's allogeneic HSCT was performed with unmanipulated bone marrow (2.4×10^6 CD34-positive cells/kg) from his HLA-identical sibling after conditioning with cyclophosphamide (CYC, 50 mg/kg/day for 4 days) and antithymocyte globulin (ATG, 15 mg/kg/day for 4 days). On day (day) −4, a first febrile episode in neutropenia occurred due to a bloodstream infection by *Pseudomonas aeruginosa*. The patient was successfully treated with piperacillin/tazobactam and tobramycin. Antimicrobial prophylaxis included aciclovir (post-transplant switched to foscarnet because of cytomegalovirus-positivity of donor and recipient) and micafungin (1×50 mg). For prophylaxis of GvHD, methotrexate (10 mg/m^2/day on day +2, +4, +7) and ciclosporin A (CSA) were administered. On day +23, CSA was replaced by tacrolimus because of peripheral neuropathy. On day +8, the patient developed a second episode of fever with rising inflammatory markers. His blood cultures remained sterile. The patient's antibacterial treatment was switched to meropenem and teicoplanin resulting in rapid resolution of the fever. Two days later, a painful fissure between the 4th and 5th left toe appeared without any physical injury. Microbiological samples revealed the presence of multisensitive *Escherichia coli*, so the antibacterial treatment was maintained. The lesion became necrotic and cavernous with a maximum depth of 1.5 cm. On day +15, painful, erythematous skin nodules were found affecting the entire integument. As infectious metastases of fungal origin were suspected, micafungin was switched to liposomal amphotericin B (LAmB, 1×3 mg/kg, from day +31 5 mg/kg, stop date day +34) and intravenous (i.v.) voriconazole (VCZ, loading dose 2×6 mg/kg, maintenance therapy 2×4 mg/kg).

Several cutaneous biopsies were taken on day +17, which histologically proved hyalohyphomycosis, compatible with invasive aspergillosis. Serum Aspergillus-galactomannan antigen on day +21 was negative. One of the formalin-fixed paraffin-embedded (FFPE) skin biopsies was sent to the Mycologic Laboratory at the Robert Koch Institute Berlin for further analysis.

Histopathology after Grocott's methenamine silver (GMS) stain showed vascular invasion and infarction by strongly stained small, septated hyphae with acute angle branching and small, single-celled chlamydoconidiae (**Figures 1A,B**). DNA was extracted by the protocol described by Rickerts et al. (4) and studied by five different qPCRs. To document successful DNA extraction a qPCR detecting the human 18S rRNA gene was used (5). An internal amplification control DNA (IAC qPCR) was used to exclude PCR inhibition. Fungal DNA was amplified using a broad range 28S qPCR (primer 10F and 12R) (5–8), and the DNA was sent to a collaborating laboratory for two specific qPCR assays to detect *Aspergillus* DNA (7, 9, 10) and DNA of *Mucorales* (7, 11). No inhibition, defined as a delta CT of more than two cycles (5), was detected. The broad range qPCR amplified fungal DNA in duplicates in less than 40 cycles (CTs) with identical peaks in the melting curve analysis in the absence of positive no-template-, or extraction negative controls. The identification of the resulting sequences by BLAST search in GenBank (12) yielded an identity of 99.7% with the sequence of an ATCC *Fusarium solani* (accession number FJ345352) strain. Both the *Aspergillus* and *Mucorales* qPCRs were negative.

The patient's first VCZ level on day +21 6 days after the start of treatment was 12 mg/l. However, multiple further tests did not detect VCZ levels above 2 mg/L despite continuous

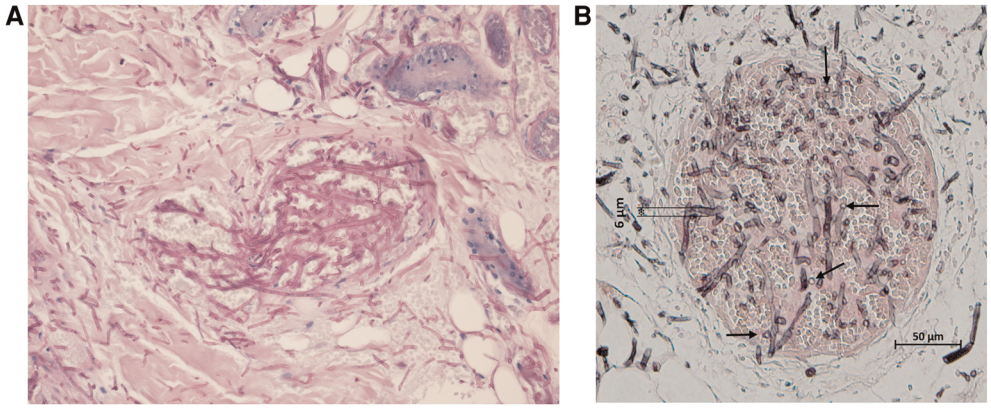

FIGURE 1
(A) Microscopic image of a skin lesion. Histologic examination of a skin biopsy following periodic acid–Schiff (PAS) staining shows the extravasation of hyphae from a blood vessel into the surrounding tissue. It is important to note that there is not any reaction of the cellular immune system against this fungal invasion. (B) Tissue biopsy of the cutaneous lesion showing vascular invasion and infarction by small, septated hyphae intermingled with chlamydoconidiae (indicated by the arrows) stained by Grocott's methenamine silver stain (magnification 10x), both day +17. Both images were obtained from the same FFPE (formalin-fixed paraffin embedded) tissue.

intravenous administration of VCZ. To exclude false-negative results, VCZ tests were performed in two different laboratories revealing identical findings. After obtaining these low trough levels, VCZ was increased to 12 mg/kg i.v. until the patient's discharge to his home.

Neutrophil engraftment with full donor chimerism was documented on day +22. Massive capillary-leak syndrome with lung and peripheral edema and acute renal failure caused by the interaction of tacrolimus and i.v. VCZ required continuous venovenous hemodialysis from day +27 to +30 and invasive mechanical ventilation due to uremic encephalopathy. Despite these transient, however serious organ failures, the patient's antimycotic treatment was maintained without interruption with i.v. VCZ. Cultures of tracheal samples and wound swabs during this first period in the pediatric intensive care unit grew hyphomycetes, which were identified as *Fusarium* species. An antifungal susceptibility testing (AFST) was done and showed sensitivity to VCZ and resistance to LAmB.

Besides the cutaneous foci, ultrasound and computed tomography (CT) also showed mycotic lesions in his lungs, liver, both kidneys, and most impressively, in the patient's spleen. The magnetic resonance imaging (MRI) of his brain and an ophthalmological examination were unremarkable. On day +46, a sudden circulatory failure resulted in a life-threatening emergency caused by a splenic rupture as demonstrated in an abdominal CT scan. Most likely, the event was caused by an initial hemorrhage into one of the mycotic lesions of the spleen, followed by the rupture of the splenic capsule. A splenectomy was performed immediately. Diffuse involvement of the spleen with mold species was confirmed upon pathological examination (**Figures 2A–C**).

The patient's condition gradually improved; his skin lesions healed with residual small scars. However, several episodes of fever were noticed from day +48 until his discharge on day +69. Blood cultures and viral monitoring remained unremarkable. Imaging studies (MRI, CT, transthoracic echocardiography) did not reveal new findings. An episode of upper abdominal pain was caused by biliary tract obstruction, which was solved by an endoscopic procedure. The patient was discharged on day +69. He was re-admitted on day +72 because of reduced general condition and insufficient eating and drinking. Supportive treatment was initiated. On day +76, the patient experienced sudden right-sided hemiparesis caused by pontine ischemia. In transesophageal echocardiography (TEE), a floating thrombus in the outflow tract of the left ventricle with a size of 1.5 cm was found (**Figures 3A,B**). A multi-disciplinary case discussion was initiated. Considering the severe previous organ failures, a surgical procedure was not favored at first. The patient received low-molecular-weight heparin and broad empirical antibacterial treatment in addition to his antifungal therapy. He continued to have regular fever spikes; blood cultures remained sterile. In a follow-up TEE 7 days later, the size of the thrombus had increased. MRI revealed a second ischemic area in the cerebellum which was asymptomatic. The patient's situation was discussed with the patient, his parents, and each involved medical department. A cardiac surgical procedure with thrombectomy was regarded as unavoidable. Although the patient acknowledged the risk of the procedure, he clearly wished to take this option as he increasingly suffered from his condition. Despite broad analgesic treatment, including opioids, he complained about persistent, severe headaches and, in addition, felt miserable because of

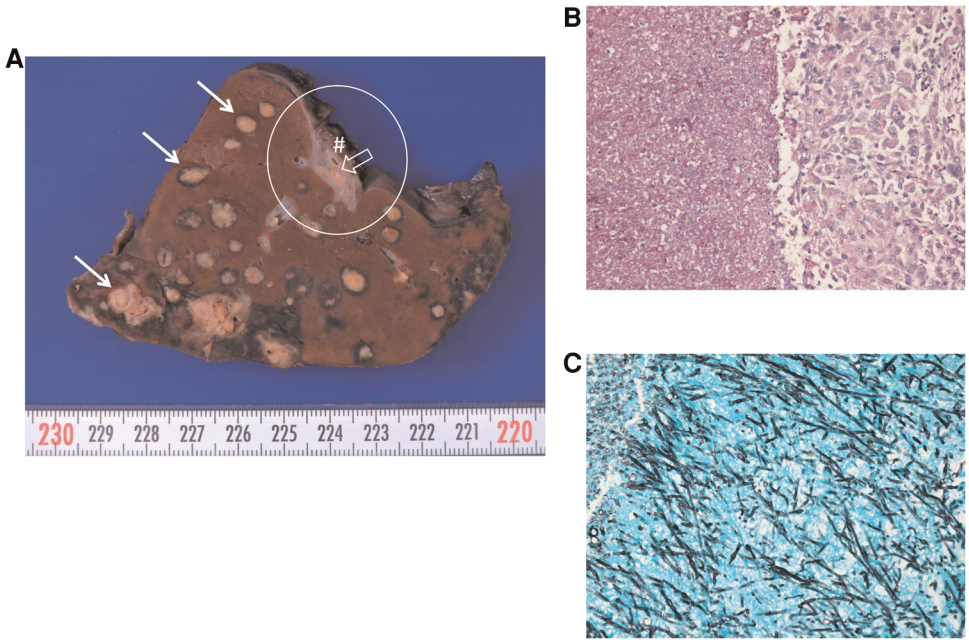

FIGURE 2
Macroscopic and microscopic image of the ruptured spleen. (A) Macroscopic image of the spleen. Solid arrows indicate multiple intravascular fungal lesions with perifocal bleeding. Asterisk shows the red pulp with dark brown discoloration consistent with transfusion associated hemosiderosis. Circle shows the splenic hilum with the lienal vein (hash sign), where fungal lesions outside the spleen can be identified (open arrow). (B,C) Microscopic images of a splenic lesion following periodic acid–Schiff (PAS). (B) and Grocott's silver staining (C) showing hyphae and chlamydoconidiae of *Fusarium solani*. The length of the lower border is 612 µm each.

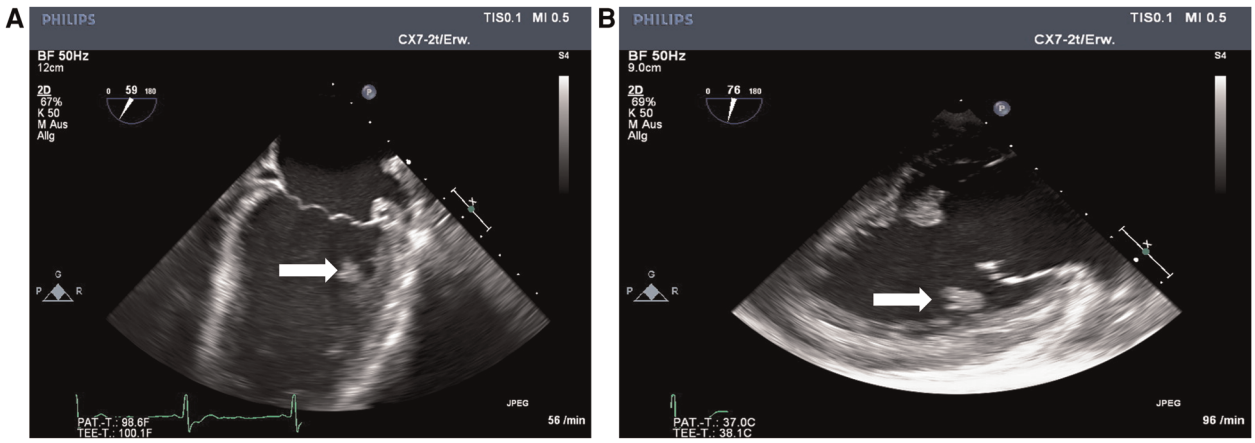

FIGURE 3
(A and B) Views on thrombus (white arrows) in the outflow tract of the left ventricle, day +80.

his unchanged hemiparesis. After the removal of the left ventricular thrombus on day +91 in a nearby cardiac surgery clinic, the patient did not regain consciousness. A CT scan revealed diffuse bilateral ischemia and edema in the brain. With respect to the expected poor neurological outcome, the parents decided not to proceed with a neurosurgical procedure. On day +92, the patient was disconnected from the respirator and died. The timeline of major events is provided in Figure 4.

A post-mortem examination was not permitted. Microbiological investigation of the thrombus confirmed the presence of *Fusarium solani* by culture. In a family conference a few months after the patient's death, the parents expressed their regrets about giving their consent to their

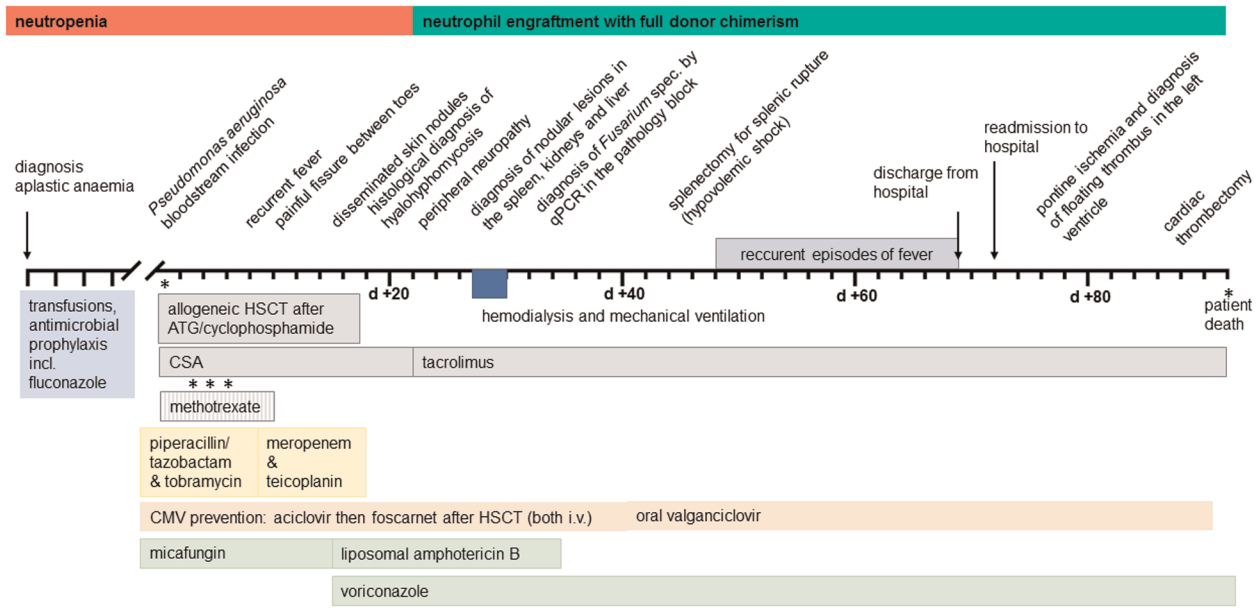

FIGURE 4
Synopsis of the clinical course and treatment. HSCT, hematopoietic stem cell transplantation; ATG, antithymocyte globulin; CSA, ciclosporin A; CMV, cytomegalovirus.

son's allogeneic HSCT. They felt he should have been granted more time to enter the procedure in a more stable physical condition.

Discussion

Despite continuous antimycotic, AFST-adapted treatment with VCZ, timely neutrophil engraftment after allogeneic HSCT, and two surgical procedures, this patient did not survive disseminated infection with *Fusarium solani*. It is possible that the acquisition of *Fusarium* happened through transcutaneous inoculation in the initial skin lesion between his toes. Unlike aspergillosis, cutaneous involvement has been described in around 85% of reported cases of *Fusarium* infection, particularly in immunosuppressed patients (13). Although the cutaneous lesions healed during treatment with VCZ and in the presence of neutrophils, the spleen remained most severely affected. This may be caused by the specific architecture of this organ. A splenic rupture due to *Fusarium* infection has not been described before. The patient's death was finally caused by embolic events in the brain originating from a thrombus in the outflow tract of the left ventricle with proven colonization by *Fusarium solani*.

Fusarium spores are widely distributed in soil, and air (3), but also in plumbing systems (14) and may cause infections in plants, animals, and humans (14–16). In most cases, the principal portal of entry are the airways, followed by skin and mucosal membranes (3). In HSCT recipients, the risk is highest in recipients of mismatched unrelated donor allogeneic transplants (17). Risk factors for invasive fusariosis in the early phase of allogeneic HSCT include the receipt of ATG, and in the late phase, nonmyeloablative conditioning regimen, GvHD, and previous invasive mold disease (2). Typically, the affected patients present with persistent or recurrent fever despite broad-range antibiotic therapy and the sudden appearance of multiple skin lesions, pneumonia, or sinusitis. Lung CT scan and cutaneous biopsies are indicated, and laboratory diagnosis relies mostly on the cultural isolation of the fungus from blood or skin lesions. Differential diagnosis with aspergillosis may be challenging, as both hyalohyphomycoses are histologically almost undistinguishable and serum galactomannan may be positive in both infections (18, 19). Current guidelines recommend treatment with VCZ (20). As the persistence of severe immunosuppression, particularly neutropenia, is the most important factor associated with the poor outcome of patients with invasive fusariosis, reduction or withdrawal of immunosuppression is recommended as early as possible.

In our patient, the diagnosis of infective endocarditis was maintained following the modified Duke criteria (21). Endocarditis caused by *Fusarium* species is rarely seen, and associated with a high mortality rate (22–25). Treatment includes long-term antifungal therapy and, if possible, surgery (26, 27). Few publications have reported this clinical manifestation before, the largest by Inano et al. in 2013 (22).

In his retrospective analysis, five of seven affected patients did not survive, demonstrating the poor prognosis of this particular site of infection. Of the two surviving patients, one had a surgical resection, the second did not. In their paper, the authors suggest the combination of VCZ with terbinafine (TBF) based on in-vitro-studies and their experience in an own patient. Randomized clinical trials to answer this question are not yet available. In our patient, it remained unclear when exactly the endocarditis developed and whether the splenic rupture contributed to the dissemination of *Fusarium* species into other organs.

As *Fusarium* is intrinsically resistant to a broad range of antifungals, and the optimal treatment strategy remains a major challenge, AFST may be a helpful tool to guide the treatment (16, 28). The global guidelines recommend as first-line treatment VCZ with therapeutic drug monitoring (TDM) or LAmB (28). When using VCZ it must be considered that low or even unmeasurable plasma levels can often be found. This is partly due to ultrafast metabolizers (29), but essentially this phenomenon is not completely understood. Some retrospective studies have identified a relationship between VCZ trough concentrations and clinical outcome (30–32), some others have not (33–35). Therefore, treatment of these often critically sick patients with disseminated mold disease may be complicated by the lack of reliable data. Different combination therapies, as VCZ plus TBF (22), CAS plus amphotericin B deoxycholate (dAmB) (36), dAmB plus VCZ (37–39) or dAmB plus TBF (40) have been described in limited case reports. More recent data justify the initial combination of VCZ with TBF (22), CAS, micafungin, or posaconazole, particularly until the targeted range of VCZ trough concentration is achieved (20). From the time a fungal infection was suspected, our patient had uninterrupted treatment with VCZ according to the recommended dosing schedule in the drug's medical specialist information sheet. Apart from 3 days in the period from day +16 until day +69, he was treated with the intravenous solution of VCZ with a daily dose of 8-12 mg/kg body weight. Surprisingly, trough levels >2 mg/L were measured only once, perhaps indicating a rapid metabolization of VCZ in this patient. In the sample obtained from the cardiac thrombus, the minimal inhibitory concentration (MIC) of the *Fusarium* species was >8 mg/L for VCZ and also for isavuconazole, as determined by the German Reference Center for Invasive Mycotic Infections. This MIC was far beyond the generally accepted VCZ trough concentration of 2–5 mg/L. Posaconazole had previously been found resistant in this patient.

Allogeneic HSCT in patients with VSAA is usually associated with a favorable outcome of 80% overall survival, particularly when using bone marrow from an HLA-identical sibling (41). Several factors may have contributed to the death of the patient reported here. We assume that the critical events were the long period of neutropenia before his allogeneic HSCT in combination with the colonization by *Fusarium* species in the rural area where he grew up. From the time of diagnosis, he regularly needed packed red cell and platelet transfusions. Although the patient noticed these symptoms and therapeutic measures due to his bone marrow failure syndrome, the elevated risk for infections was not quite perceivable and delayed the decision to proceed to bone marrow transplantation.

In this case report, many questions remain unsolved and limit the reliable assessment of all events. First, it was not possible to define the time of colonization with the *Fusarium* species. Pre-transplant microbiological tests included routine screening for multi-resistant bacteria and swabs from the throat, but not swabs from other regions of the body. We think that colonization took place in the domestic area with close contact to animals and grain. A source inside the hospital appears unlikely, as there have been no further infections by *Fusarium* species in the past 10 years. Second, although splenic rupture was a major event and has not been reported before in patients with fusariosis, the most critical condition was the development of fungal endocarditis with subsequent embolism into the brain. Neither persistent treatment with VCZ nor the presence of neutrophils was able to prevent these complications. Third, it remains a matter of discussion whether higher doses of VCZ, a switch to isavuconazole or a transesophageal echocardiography at an earlier date would have resulted in a different outcome.

In conclusion, despite the uninterrupted administration of antimycotic substances, disseminated fusariosis remains a challenging and life-threatening condition in immunocompromised patients after allogeneic HSCT. Uncommon manifestations of this disease need to be considered at any time during the transplant procedure. Affected patients should undergo thorough examinations, including CT scans, MRI of the brain, ultrasound, funduscopy, and particularly echocardiography, which allows a reliable assessment of the heart valves.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Ethikkommission an der Universitätsmedizin Greifswald Institut für Pharmakologie Felix-Hausdorff-Str. 3 17487 Greifswald. Written informed consent to participate in

this study was provided by the participants' legal guardian/next of kin.

Author contributions

MH, DW and KE wrote and supervised the manuscript. FD, JL, AK, AH, LU, JB, VR, JS and HL provided significant information. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case Report: Pediatric myeloid/lymphoid neoplasm with eosinophilia and PDGFRA rearrangement: The first case presenting as B-lymphoblastic lymphoma

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According to the latest WHO classification of hematopoietic malignancies, myeloid and lymphoid neoplasms with eosinophilia and gene rearrangements include three specific rare diseases and one provisional entity. Myeloid/lymphoid neoplasms with platelet-derived growth factor receptor alpha (PDGFRA) rearrangements are the most frequent of these disorders and are usually present in adult males with a median age of the late 40s. Patients usually have chronic eosinophilic leukemia but can occasionally manifest as acute myeloid leukemia or extramedullary T- or B-lineage lymphoblastic lymphoma. We report a case of a previously healthy 2-year-old girl who presented with a right supraorbital swelling with no associated lymphadenopathy. Peripheral blood smear evaluation at initial presentation revealed microcytic hypochromic red blood cells and leukocytosis with marked eosinophilia, occasional myelocytes, and occasional blasts. Whole-body CT scans and PET scans revealed hypermetabolic potentially lymphomatous mass in the superior medial aspect of the right orbit in addition to splenomegaly but no evidence of hypermetabolic mediastinal, hilar, abdominal, or pelvic lymph nodes. Bone marrow aspirate and biopsy revealed hypercellular bone marrow with quantitatively decreased erythroid precursors and increased granulocytic precursors with 60% of the cells being eosinophilic cells in different stages of maturation. The diagnosis of myeloid neoplasm with eosinophilia and rearrangement of PDGFRA was made following confirmation by fluorescence *in situ* hybridization (FISH) test for *FIP1L1-PDGFRA* gene fusion. An incisional biopsy of the supraorbital mass revealed B-cell lymphoblastic lymphoma (B-LBL). FISH test for *FIP1L1-PDGFRA* gene fusion was positive in 70% of the cells studied. Thus, the final diagnosis was B-cell lymphoblastic lymphoma arising in the setting of myeloid/lymphoid neoplasm with eosinophilia and PDGFRA rearrangement. The patient was started on imatinib with concomitant therapy for B-LBL per the Children Oncology Group (COG) standard therapy for localized B-LBL and demonstrated a favorable outcome in the 2.5-year follow-up period. To our knowledge, this is the first pediatric

case of myeloid/lymphoid neoplasm with PDGFRA rearrangement presenting with synchronous myeloproliferative disease and B-LBL. We present our diagnostic and management approach of this patient and review prior relevant pediatric cases of myeloid/lymphoid neoplasms with PDGFRA rearrangement.

KEYWORDS

myeloid/lymphoid neoplasms, eosinophilia, *PDGFRA*, B-cell lymphoblastic lymphoma (B-LBL), imatinib

Introduction

Myeloid/lymphoid neoplasms with eosinophilia (M/LN-Eo) and rearrangements of *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2* genetic variants constitute a rare but well-defined category of hematologic malignancies recognized by the WHO revised classification (1). Despite having myeloproliferative neoplasm (MPN) and eosinophilia as common manifestations in most reported cases, individual patients may display remarkable heterogeneity at initial presentation and clinical course (2). Myeloid/lymphoid neoplasms with PDGFRA rearrangements are the most frequently encountered of these disorders and are usually present in adult males with a median age of the late 40s (1–3). Patients usually have chronic eosinophilic leukemia (CEL) but can also manifest as acute myeloid leukemia (AML), systemic mastocytosis (SM) with hypereosinophilia, or extramedullary T- or B-lineage lymphoblastic lymphoma (T-LBL/B-LBL) (2). Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangements are extremely rare in the pediatric population with only few cases reported in the literature.

Given its exceptional therapeutic benefit demonstrated in previous reports, the tyrosine kinase inhibitor imatinib is currently considered first-line therapy for patients with M/LN-Eo harboring rearrangements of *PDGFRA* (2). However, due to the rarity of these diseases in the pediatric population, and the lack of reports describing imatinib monotherapy in cases presenting with T-LBL/B-LBL, treating physicians are often left with challenging decisions when formulating their management plans. In this report, we present the first pediatric case of myeloid/lymphoid neoplasm with *PDGFRA* rearrangement presenting with synchronous myeloproliferative disease and B-cell lymphoblastic lymphoma (B-LBL). We present our diagnostic and management approach of this patient and review prior relevant pediatric cases of myeloid/lymphoid neoplasms with *PDGFRA* rearrangements.

Case report

A previously healthy 2-year-old girl presented with right supraorbital swelling that developed over 1.5 months with no other symptoms at the time of initial presentation. Notable on examination was a right firm supraorbital swelling causing ptosis, with no overlying skin changes (**Figure 1A**). Examination was also notable for hepatosplenomegaly but not lymphadenopathy. Initial laboratory data were significant for anemia (Hb 8.2 g/dl) and leukocytosis (WBC 19.5×10^9/L) with eosinophilia of 32% and an absolute eosinophilic count of 6.3×10^9/L. Blood smear showed microcytic, hypochromic red blood cells (RBCs) in addition to leukocytosis with marked eosinophilia (32%), occasional myelocytes (2%), and occasional myeloblasts (less than 1%). Some of the eosinophils had three to four nuclear segments and polarized distribution of the cytoplasmic granules.

Whole-body PET/CT scan revealed hypermetabolic potentially lymphomatous mass in the superior medial aspect of the right orbit in addition to splenomegaly, but no evidence of hypermetabolic mediastinal, hilar, abdominal or pelvic lymph nodes. MRI of the orbits revealed a lobulated well-defined nonhomogenous enhancing mass in the superior medial aspect of the right orbit measuring 2.2 cm × 0.6 cm × 2.8 cm. The lesion was mostly involving the anterior aspect of the medial rectus muscle, causing destruction of the medial aspect of the right orbital roof with mild extra-axial intracranial extension but no compression of the brain parenchyma. There was a mild extension of the subcutaneous tissue in the supraorbital space, with compression of the right eye globe but no evidence of invasion of the sclera or retro-ocular extension (**Figure 2A**).

Bone marrow (BM) aspirate and biopsy revealed hypercellular BM with quantitatively decreased erythroid precursors and quantitatively increased granulocytic precursors with 60% of the cells being eosinophilic cells in different stages of maturation. Megakaryocytes were quantitatively normal (**Figure 3A**). Flow cytometry showed less than 1% myeloblasts. No B-cell lymphoblasts were detected. The diagnosis of myeloid neoplasm with eosinophilia and rearrangement of *PDGFRA* was made following confirmation by fluorescence *in situ* hybridization (FISH) test for *FIP1L1-PDGFRA* gene fusion. Cerebrospinal fluid (CSF) examination showed an acellular specimen with no blast or atypical cells. An incisional biopsy of the supraorbital mass revealed B-cell lymphoblastic lymphoma (B-LBL) (**Figure 3B–D**). FISH test for *FIP1L1-PDGFRA* gene fusion was positive in 70% of the cells studied (**Figure 3E**).

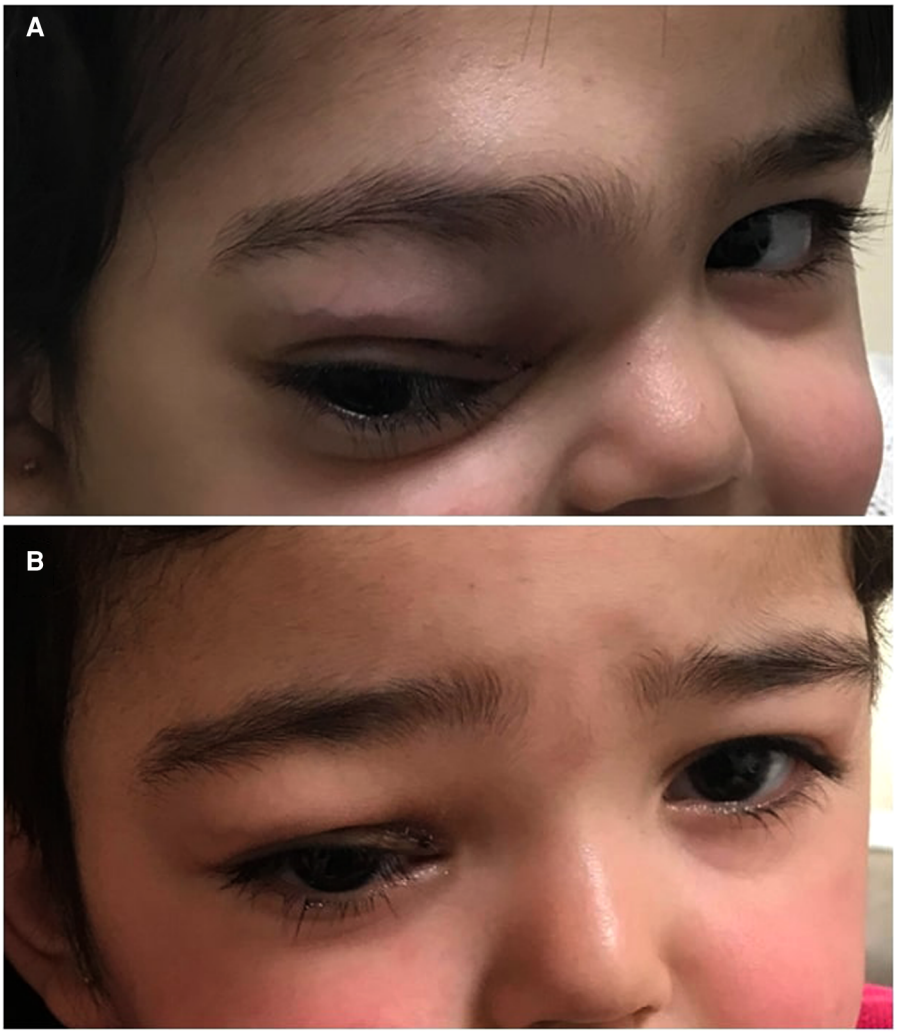

FIGURE 1
(**A**) Right supraorbital swelling at initial presentation. (**B**) Resolved right supraorbital swelling following 5 months of combined chemotherapy with imatinib.

Thus, the final diagnosis was B-cell lymphoblastic lymphoma arising in the setting of myeloid/lymphoid neoplasm with eosinophilia and *PDGFRA* rearrangement.

Cardiac evaluation was normal. Gastrointestinal evaluation that included upper endoscopy and colonoscopy showed chronic gastric, duodenal, and rectal inflammation but no evidence of eosinophilic infiltration or B-cell lymphoma.

The patient was started on imatinib 340 mg/m^2/day with concomitant therapy for B-LBL per the Children Oncology Group (COG) standard therapy for localized B-LBL. WBC count and absolute eosinophilic count normalized after 4 days of imatinib. One month after the initiation of therapy, which corresponded to the end of chemotherapy induction phase, the supraorbital swelling decreased grossly to 50% of its original size. The BM evaluation was morphologically normal and the *FIP1L1-PDGFRA* gene fusion by FISH analysis was decreased to 2.5%. Five months following treatment, the supraorbital swelling had completely resolved (**Figure 1B**). Whole-body PET CT showed almost complete metabolic response, and the brain and orbital MRI showed almost complete resolution of the right orbital mass (**Figure 2B**). The BM evaluation was also in morphologic remission with negative FISH for *PDFGRA* rearrangement. After 2.5 years of treatment, the multiagent chemotherapy for B-LBL was completed and the patient is now on imatinib monotherapy with close follow-up.

Discussion

Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangements are extremely rare in the pediatric population

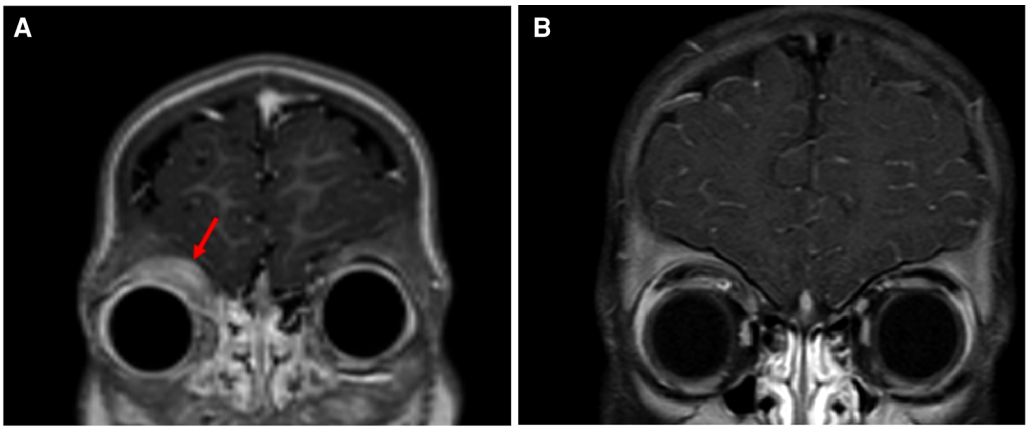

FIGURE 2
(A) Orbital MRI (coronal T1 fat sat postcontrast section) at initial presentation showing mass in the superior medial aspect of the right orbit (arrow). (B) Orbital MRI (Coronal T1 fat sat postcontrast section) following 5 months of combined chemotherapy with imatinib demonstrates marked improvement of tumor size.

with only few cases reported in the literature. To the best of our knowledge, only a few pediatric cases were reported describing myeloid/lymphoid neoplasms harboring *PDGFRA* rearrangements (4–14) (**Table 1**).

Despite the well-known and very strong male predominance in adult cases of hypereosinophilic syndromes (HES), in general, and myeloid/lymphoid neoplasms with *PDGFRA* rearrangements, in particular, pediatric cases demonstrate a less pronounced

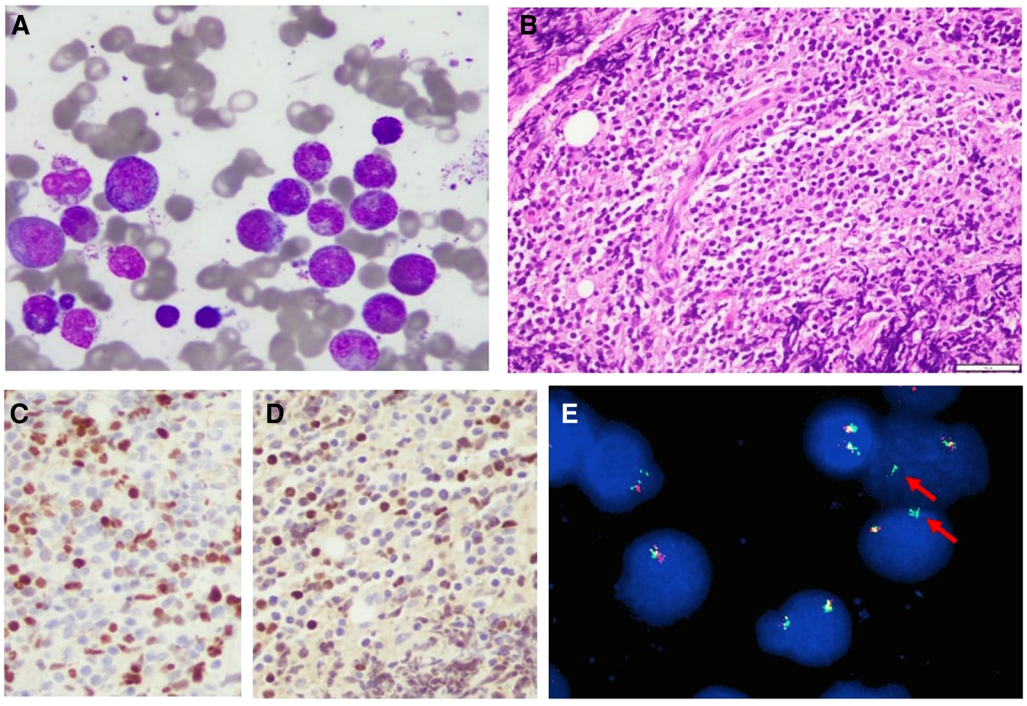

FIGURE 3
(A) Bone marrow aspirate showing mostly eosinophils, Wright–Giemsa stain, 40×. (B) Biopsy from the orbital mass demonstrating heavy lymphoid infiltrate composed of small- to medium-sized lymphoblasts, hematoxylin and eosin stain, 40×. (C,D) PAX5 and TdT immunostains showing positive brown nuclear stain in the tumor cells, respectively, indicating that these cells are B-cell lymphoblasts 40×. (E) Rearranged PDGFRA gene (arrows). Rearrangement results from fusion of FIPL1 and PDGFRA after CHIC2 deletion.

TABLE 1 Summary of reported pediatric cases of myeloid/lymphoid neoplasms with *FIP1L1-PDGFRA* rearrangement.

Reference	Age (years)	Gender	Clinical features at presentation	Initial WBC count ($\times 10^9$/l)	Initial eosinophilic count ($\times 10^9$/l)	Disease type	Imatinib dose	Other treatment lines used	Time until achieving hematologic and molecular remission		Follow-up/Long-term outcome
									CHR	CMR	
Rives et al. 2005 (4)	7	Male	Incidental eosinophilia detected on a preoperative blood followed by generalized pruritus and malaise	13.5	6.1	Chronic MPN with eosinophilia/ CEL	–	Prednisone	1 month	Not reported	Relapsed after corticosteroids discontinuation but responded to resuming treatment with prednisone, which was maintained for 6 months. Authors intend to treat with imatinib in case of relapse.
Rathe et al. 2010 and 2014 (5, 6)	2	Female	Malaise, fatigue, loss of appetite, and pain	125.0	22.5	Chronic MPN with eosinophilia/ CEL	300 mg/m^2 daily	—	2 weeks	3 months	Continued imatinib for 5 years. Five months after discontinuing therapy, the patient relapsed and imatinib was restarted. CHR and CMR were reached at 4 weeks.
Rapanotti et al, 2010 (7)	16	Male	Lymphadenopathy, splenomegaly, restrictive cardiomyopathy	13.5	5.3	Chronic MPN with eosinophilia/ CEL	400 mg daily	—	Achieved with imatinib, timeline not specified		Remained in remission for 36 months
Farruggia et al. 2014 (8)	14	Male	Pallor, weight loss (3 kg in 2 months), and left shoulder pain, lymphadenopathy, hepatosplenomegaly	131.1	49.0	Chronic MPN with eosinophilia/ CEL	200 mg daily	Hydroxycarbamide treatment—initiated prior to identifying gene rearrangement—did not control the disease	40 days	8 months	Remained in remission for 12 months
Zeng et al. 2015 (9)	9	Female	Malnutrition, fever, cough, diarrhea, lymphadenopathy, hepatosplenomegaly, progressive skin rash starting at 1 month of age, bilateral keratomalacia with corneal erosions at 3 years, stroke at 7 years, cardiac dysfunction, pneumatosis effusion along within the intestine tract	39.7	28.9	Chronic MPN with eosinophilia/ CEL	100 mg daily	Corticosteroid treatment —initiated prior to identifying gene rearrangement—did not control the disease. It was gradually tapered and withdrawn	6 weeks	Not reported	Improved end organs functions at 6 weeks

(*continued*)

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TABLE 1 Continued

Reference	Age (years)	Gender	Clinical features at presentation	Initial WBC count ($\times 10^9$/l)	Initial eosinophilic count ($\times 10^9$/l)	Disease type	Imatinib dose	Other treatment lines used	Time until achieving hematologic and molecular remission		Follow-up/Long-term outcome
									CHR	CMR	
Oberley et al. 2017 (10)	13	Male	Enlarged right supraclavicular lymph node	1.4	0.0	**T-cell lymphoblastic leukemia/ lymphoma**	400 mg daily (added on relapse)	Received standard chemotherapy for T-LBL but had a refractory disease. Imatinib was added after PDGFRA rearrangement was detected. The patient did not respond and died of disease progression at day +217 from diagnosis			
Srinivasan et al. 2019 (11)	15	Male	Migrating joint pain, and leukocytosis, splenomegaly	49.2	16.7	Chronic MPN with eosinophilia/ CEL	100 mg daily switched to maintenance dose of 200 mg weekly at around 18 months	Initially started on hydroxyurea prior to identifying gene rearrangement	1 month	3 months	Remained in remission for 42 months since diagnosis and doing well on maintenance imatinib
Bota et al. 2019 (12)	5	Female	Constitutional symptoms, multifocal bone pain, headache, gastrointestinal complaints, hepatosplenomegaly, lymphadenopathy cardiomyopathy	59.7	29.4	Chronic MPN with eosinophilia/ CEL	100 mg daily (~133 mg/m^2/ day) Switched to 100 mg every other day (~54 mg/m^2/ day) at 9 months	Concomitant methylprednisolone (1 mg/kg/day for 14 days)	3 days	9 months	Remained in remission for 16 months. Cardiomyopathy completely resolved
Jain et al. 2020 (13)	6	Male	Fever, fatigue, massive splenomegaly	18.5	6.1	Chronic MPN, blast phase (23% BM blasts)	100 mg daily (~100 mg/m2/ day)	—	6 weeks	19 weeks	Remained in remission for 2 years since diagnosis
Voeller et al. 2020 (14)	13	Female	Iron deficiency, anorexia, anxiety, frequent panic attacks and exertional shortness of breath, severe restrictive cardiomyopathy with pulmonary hypertension	Not reported—	4–6	Chronic MPN with eosinophilia/ CEL	100 mg daily	Steroids	1 month	3 months	Remained in remission after nearly a year on continued monotherapy with imatinib. Remained asymptomatic from a cardiovascular standpoint, but continued to have pulmonary hypertension and severe diastolic dysfunction
Current case	2	Female	Right supraorbital swelling, hepatosplenomegaly	19.5 6.3	6.3	**B-cell lymphoblastic lymphoma**	~340 mg/m^2/day	Concomitant therapy for B-LBL per the COG standard therapy protocol	4 days	5 months	Remained in remission for 2.5 years since diagnosis

WBC, white blood cell; CHR, complete hematologic remission; CMR, complete molecular remission; MPN, myeloproliferative neoplasm; CEL, chronic eosinophilic leukemia; COG, children oncology group; PDGFRA, platelet-derived growth factor receptor alpha.

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male predominance (13, 15). In adults, patients with M/LN-Eo with *PDGFRA* rearrangements usually present as CEL, but can rarely manifest as AML, SM with hypereosinophilia, or extramedullary T-LBL/B-LBL (2). All previously reported pediatric cases manifested as chronic MPN with eosinophilia/CEL with only one case presenting as T-lymphoblastic lymphoma (T-LBL) without eosinophilia (10). To the best of our knowledge, we report the first pediatric case of myeloid/lymphoid neoplasm with *PDGFRA* rearrangement presenting with synchronous myeloproliferative disease and B-lymphoblastic lymphoma (B-LBL).

The product of the *FIP1L1-PDGFRA* fusion gene is a constitutively activated tyrosine kinase, thus, making the tyrosine kinase inhibitor imatinib a well-known agent for targeting conditions with this specific genetic abnormality (16). Imatinib is currently considered the first-line therapy for patients with M/LN-Eo harboring rearrangements of *PDGFRA* (2). In 2005, Rives et al. reported the first child with HES/CEL harboring *FIP1L1-PDGFRA* rearrangement. However, this genetic abnormality was not identified at initial presentation. The patient had received corticosteroids for the diagnosis of primary hypereosinophilic syndrome and achieved complete hematologic response in 1 month. The patient relapsed after discontinuation of the corticosteroids but responded to resuming same treatment. After the *FIP1L1-PDGFRA* fusion gene was identified, the diagnosis was modified to *FIP1L1-PDGFRA*-positive CEL and the authors declared the intent of initiation of imatinib if their patient exhibits a second relapse (4). All subsequent reported pediatric cases included imatinib in their treatment plan (5, 7–14). Favorable outcomes were reported in all cases except the case presenting as T-LBL that was reported by Oberley et al. in 2017 (10). Their 13-year-old male patient had chemotherapy-resistant T-LBL, and once the *FIP1L1-PDGFRA* rearrangement was detected, imatinib was added. However, the patient did not respond and eventually died of his disease (10). Our patient was treated with both imatinib and standard chemotherapy for the B-LBL due to the lack of reports describing imatinib monotherapy in cases presenting with T-LBL/B-LBL in all age groups, in addition to the serious initial presentation of B-LBL that posed an imminent threat to the patient's vision and central nervous system as evident by the aforementioned imaging findings. Five months following treatment, our patient achieved complete clinical, hematologic, and molecular remission. After 2.5 years of treatment, multiagent chemotherapy for B-LBL was completed and the patient is now on imatinib monotherapy and close follow-up.

Discontinuation of imatinib has been associated with relapse in *FIP1L1/PDGFRA*-positive chronic eosinophilic leukemia in both adult and pediatric cases (6, 17, 18). Although few authors reported maintained remission after decreasing imatinib maintenance dose, there is a lack of consensus regarding the optimal maintenance duration (11, 12). It is advised that patients remain on regular follow-up by molecular monitoring to dictate optimal duration of continuation therapy. Moreover, it is generally presumed that patients may require lifelong therapy (6, 13).

Conclusion

Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangements are extremely rare in the pediatric population with only few cases reported in the literature. In this report, we presented the first pediatric case of myeloid/lymphoid neoplasm with *PDGFRA* rearrangement presenting with synchronous myeloproliferative disease and B-LBL. Our patient demonstrated a favorable outcome after initiating combined chemotherapy with imatinib.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case report: "Congenital cutaneous langerhans cell histiocytosis presenting with blueberry Muffin Rash"

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Congenital cutaneous Langerhans cell histiocytosis-(LCH), named Hashimoto Pritzker disease, is a rare subtype among the clinical spectrum of LCH that often presents at birth or through the neonatal term and spontaneously resolve within a few months. In rare instances, infants with congenital cutaneous LCH may present with a blueberry-muffin rash. We reported a case of a male newborn who presented with blueberry muffin rash and was diagnosed with congenital cutaneous LCH later on. The diagnosis was confirmed by excluding other possible systemic causes of blueberry muffin rash, followed by a skin biopsy. Skin biopsy showed reticular dermis-hypodermis infiltration by medium-sized cells which had a pale eosinophilic cytoplasm and irregular nuclei. The lesional cells were positive for Langerin, CD1a, S100, and CD68 immunostains, consistent with congenital cutaneous LCH. Investigations were performed and revealed no systematic disease involvement. After a discussion with the pediatric Hemato-Oncologist, the decision was to keep track of a "wait-and-see" approach. Long-term follow-up revealed no recurrence of the cutaneous lesions or any systemic involvement, which further leads to congenital cutaneous LCH diagnosis. Even though it is very rare, blueberry muffin rash differential diagnosis should include congenital cutaneous LCH. Early recognition of this condition protects patients from unnecessary and possibly unsafe systemic treatment.

KEYWORDS

langerhans cell histiocytosis (LCH), muffin rash, hashimoto pritzker disease, pediatrics, hematology, dermatology, pediatric hematological diseases

Introduction

Langerhans cell Histiocytosis (LCH) is a rare condition described initially by Lichtenstein in 1953. It is defined by clonal proliferation and accretion of Langerhans cells in different tissues resulting in organ damage or malignancy formation. Although the mechanism behind this accumulation remains uncertain, LCH is supposed to have a neoplastic or inflammatory process (1–4). LCH mainly affects the pediatric population, with wide heterogeneity in clinical presentations

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and consequences. The incidence of LCH is about 5 per million children and cutaneous involvement presents in 40% of these cases (5).

Previously, LCH was classified into four subcategories: Letterer-Siwe disease, eosinophilic granuloma, Hand-Schüller-Christian disease, and Hashimoto-Pritzker disease. However, recently it has been specified as a clinical spectrum with a new classification based on involving one or more organ systems, one or more locations within a single organ, and involvement of high-risk organs, particularly the liver, spleen, and bone marrow (4–9). Congenital cutaneous LCH is generally a benign condition involving one-system, distinguished by generalized red-brown macules, papules, and nodules. The disease rarely involves the skeleton, lungs, eyes, and abdomen (10, 11).

The term "Blueberry muffin baby" (BMB) was initially used for the description of congenital rubella cutaneous manifestations in the 1960s (12). They are characterized by multiple, non-blanching, purple to dark blue macules, papules, or nodules. Rather than rubella, various diseases have been related to BMB, including congenital infections (e.g., Toxoplasmosis, Rubella, Cytomegalovirus, Herpes and other agents (TORCH), Parvovirus), hematologic disorders, metabolic disorders, neoplastic and other diseases (i.e., Langerhans cell histiocytosis, neonatal lupus). In rare cases, Congenital cutaneous LCH manifests clinically as BMB (13, 14). Herein, we describe an unusual Congenital cutaneous LCH case, who presented with blueberry muffin rash and had an excellent clinical outcome.

Case presentation

Our patient is a male newborn who was referred to our center at the age of 4 days with a blueberry muffin rash. He was born *via* normal vaginal delivery after an uneventful full-term pregnancy for a healthy 21-year-old primigravida woman. Physical examination revealed several scattered dark purple papules-like spots over the face, neck, and limbs of variable sizes (2–4 mm) (**Figure 1**). The rash was palpable with a smooth surface. There was no hepatosplenomegaly, jaundice, or ecchymosis. He had normal physical neuroexam. Transfontanelle ultrasound for brain was done and normal. Otherwise, the baby was active and healthy. No history of bruises, bleeding, fever, or abnormality in movement. His growth parameters were between the mean and −1 Standard Deviation (SD).

Multiple Investigations were performed, including complete blood count (CBC), blood serum chemistry, coagulation profile, liver function tests, and blood film, all of which were normal. Serology for congenital infections showed the following results; VDRL: non-reactive, TORCH: negative except for high cytomegalovirus (CMV) IgG antibody, and Qualitative PCR testing for urine and blood were negative. A urine sample analysis to rule out neuroblastoma did not show homovanillic acid (HVA) or Vanillylmandelic acid (VMA). Skin biopsy revealed a reticular dermis-hypodermis infiltration by medium-sized cells, which had a pale eosinophilic cytoplasm and irregular nuclei. The cells were positive for Langerin, CD1a, S100, and CD68 immunostains, consistent with cutaneous Langerhans cell histiocytosis (**Figures 2,3**). Further investigations were performed to exclude systemic involvement, including a skeletal survey with a skull

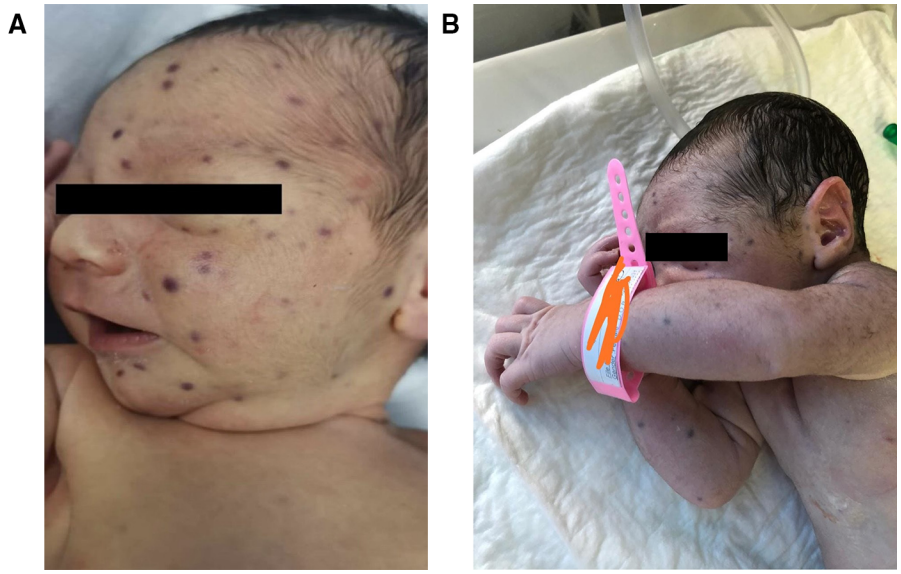

FIGURE 1
Blueberry muffin baby. (**A**) & (**B**) Disseminated dark red to purplish macules and papules on the face, neck, and upper limbs.

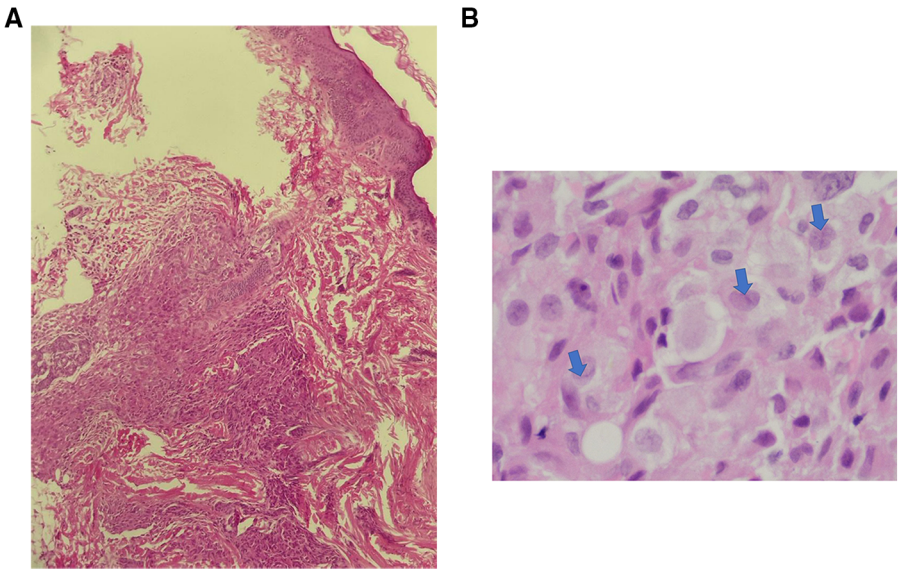

FIGURE 2
Congenital cutaneous langerhans cell histiocytosis. (**A**). Dermal- hypodermal infiltration by histocytes (H & E. 20X), (**B**). The lesional cells have abundant, light eosinophilic cytoplasm and irregular nuclei with frequent nuclear folds and grooves (arrows), obscure nucleoli and fine chromatin.

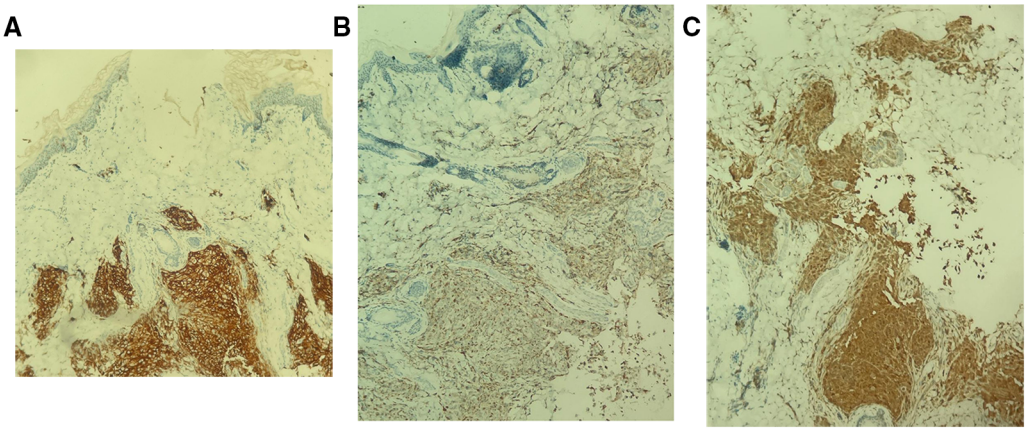

FIGURE 3
Immunohistochemistry. The lesional histocytes are diffusely positive for CD1a (**A**), CD68 (**B**), and S100 (**C**).

view, and abdominal ultrasound, all of which were normal, and supported the diagnosis of congenital cutaneous LCH.

After a discussion with the pediatric Hemato-Oncologist, a "wait-and-see" approach was preferred. The infant remained stable and active during his stay with good general condition. Labs were followed up and showed good results; the rash faded with time. He was discharged home with recommendations to follow up with a pediatric hemato-oncologist. At the age of two months, almost the whole lesions on his skin have resolved. His last follow up was at the age of two years, there was no evidence of recurrence or any extracutaneous manifestations. No continuity of follow up beyond this age.

Discussion

Blueberry muffin baby (BMB) is a clinical manifestation caused by extramedullary hematopoiesis within the dermis. Clinically, it is characterized by diffuse dark bluish to purple papules or nodules. BMB appearance had a broad spectrum of

differential diagnoses, including congenital infections (TORCH and Epstein Barr virus), malignancies/proliferative conditions (neuroblastoma, congenital leukemia, congenital rhabdomyosarcoma, LCH), hematologic disorders (rhesus hemolytic anemia, hereditary spherocytosis, ABO blood groups incompatibility, and twin-twin transfusion syndrome) (14–16). Congenital Cutaneous LCH is an infrequent presentation among the BMB differentials (17).

Congenital cutaneous LCH is considered a rare variant of single-site LCH, first reported by Hashimoto and Pritzker in 1973 (10, 11). Most cases are present at birth or later in the neonatal term with cutaneous manifestations represented by reddish-brown or violaceous papules or nodular lesions. The disease clinical course is often benign with a spontaneous – resolution tendency. However, in a few patients, the disease may progress to multi-systemic LCH affecting other organs, especially the spleen, liver, bone, and lymph node (11, 15, 16, 18).

Besides the disease's clinical course, skin biopsy demonstrates histologic and immunohistochemical findings are crucial in the diagnosis. The typical histopathological appearance of cutaneous LCH shows infiltration of the dermis by cells that have abundant, light eosinophilic cytoplasm and irregular nuclei with frequent nuclear folds and grooves, obscure nucleoli, and fine chromatin. Some inflammatory cells, such as lymphocytes, eosinophils, neutrophils, and mast cells, may coexist. Immunohistochemical stains, including S100, CD207 (langerin), and CD1a, are essential to confirm the diagnosis. The gold standard is evidence of Birbeck granules on electron microscopy (15, 16, 18).

Regarding our case, a step-wise plan was followed to investigate the cause of the patient's BMB presentation. Congenital infection was our first concern; therefore, extensive infectious workup was done, which revealed negative results except for high levels of CMV IgG anti-body. Hematological malignancies were unlikely, with a normal CBC and blood film. Also, neuroblastoma was excluded by a normal urine level of VMA and HMA. A skin biopsy confirmed the diagnosis of LCH. To look for other body systems involvement, a skeletal survey was done, including skull views as it is the most common site for LCH and it was free of any lesions. Additionally, the abdominal ultrasound was normal. The rash gradually faded and it disappeared entirely by the age of two months. His last follow-up at the age of two years has revealed no evidence of recurrence or any extracutaneous manifestations.

A comprehensive literature review on PubMed, Google Scholar, and Embase revealed 11 cases of congenital cutaneous LCH present with BMB, including the current case. All cases had spontaneous resolution of the disease during the first few weeks of life, ranging from 15 days up to 18 months without requiring any treatment. In our case, the rash disappeared at the age of two months. Follow up period in the reported cases ranging from 11 months till 2 years and none of those cases showed evidence of recurrence (2, 17, 19–22).

Since most cases of isolated cutaneous LCH has a self-limited course within a few months, the treatment is usually conservative. A careful watchful waiting approach is approved in many cases which is comparable to our case. Topical steroids may also be used in non-resolving cases. Furthermore, severe symptomatic cutaneous lesions necessitate the use of other treatment options such as topical nitrogen mustard or psoralen with ultraviolet light therapy (17, 18). Close monitoring for any signs of recurrence or progression to multisystem disease is crucial. Following the early thorough workup, all newborns should be monitored by a careful clinical examination, CBC, and possibly ultrasounds. A potentially serious multisystem disease progression might happen within several weeks to months, for which systemic therapy is indicated (16).

Our case has some limitations, lack of follow up information at time of writing the report, and parents didn't adhere to follow up in our hospital.

Conclusion

To summarize, our case highlights the importance of raising the index of suspicion toward considering congenital cutaneous LCH as a cause of BMB. A comprehensive diagnostic approach should be implemented to exclude infectious and systemic causes as well as skin biopsy findings, which are critical for the diagnosis and thus allow the wait-and-observe approach. Regular follow-up is mandatory in these patients for the detection of any possible relapses or disease progression.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

AY and MT contributed equally in writing the bulk of the article and made a significant contribution to the work. DA and HS assist in writing and communication with the

patient's parents. SB and AA participated in the coordination and acquisition of data, MA and MI conceived the study. All authors contributed to manuscript revisions, and all authors approved the final version of the manuscript and agreed to be held accountable for its content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Case report: Venetoclax therapy in a boy with acute myeloid leukemia in Shwachman Diamond syndrome

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Shwachman-Diamond syndrome (SDS) is a rare bone marrow failure syndrome characterized by exocrine pancreatic insufficiency, bone abnormalities, progressive cytopenia, and predispositions to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). AML, in these patients, is associated with a poor prognosis and with an increased risk of organ toxicity and infectious complications from chemotherapy and hematopoietic stem cell transplantation (HSCT), thus leading to high rates of treatment-related morbidity and mortality. The BCL-2 inhibitor venetoclax has revolutionized the treatment of AML in elderly adults, especially for treatment-naive elderly patients who are ineligible for intensive chemotherapy. There is limited evidence on the use of venetoclax in pediatric patients with SDS-related MDS or AML. Here, we report a case of a 14-year-old boy with SDS with AML arising from MDS. The patient was treated with two cycles of conventional chemotherapy with fludarabine and cytarabine with an initial good response but immediate relapse and substantial toxicity. Treatment with venetoclax and azacitidine was started, with a substantial reduction of leukemic burden (good response on peripheral leukemic infiltration and partial response in the bone marrow after one course). However, it was followed by multiple infectious complications and worsening of the general condition not allowing treatment to be continued, and the patient eventually died from multiorgan failure. With the limitations of observation of a single patient, our experience suggests that venetoclax/azacitidine combination therapy may represent a therapeutic possibility for patients with SDS and AML, even though it may be associated with significant toxicity.

KEYWORDS

Shwachman Diamond syndrome, venetoclax, acute myeloid leukemia, myelodysplastic syndromes, pediatric

Introduction

Pediatric myelodysplastic syndromes (MDS) are clonal affections of hematopoiesis leading to refractory cytopenia, abnormal maturation of hematopoietic precursors, and risk of evolution to acute myeloid leukemia (AML) (1). Shwachman Diamond

syndrome (SDS) is a rare autosomal recessive disease characterized by abnormal hematopoiesis, pancreatic insufficiency, and bone alterations (2, 3). Almost all children with SDS have moderate-to-severe neutropenia and about half of them will also experience some degree of anemia or thrombocytopenia due to inefficient bone marrow production (4). A real MDS may eventually ensue, affecting 19%–25% of patients at 20 years of age and up to 36%–40% at 30 years of age, with some patients evolving to AML (5). How MDS appears and subsequently progresses to AML is still not clear. The main hypotheses implicate an unbalance between pro-apoptotic and anti-apoptotic signals (such as BCL2) in the stressed bone marrow (6, 7). Moreover the SBDS protein may be particularly important in stabilizing the mitotic spindle (8). The prognosis of SDS patients with MDS/AML is dismal, and their management is challenging. Chemotherapy alone is not a feasible approach due to the difficulty of regenerating normal hematopoiesis, the high incidence of relapse or new clonal evolution, and the increased risk of toxicity (mostly infections) associated with standard AML therapeutic protocols (9, 10). As a result, the only curative option remains hematopoietic stem cell transplantation (HSCT). SDS patients are at an increased risk of transplant-related complications and mortality, including cardiac failure during conditioning with cyclophosphamide and Graft versus Host Disease (11, 12). The role of pre-transplant chemotherapy as a mean to reduce disease load is still unclear. In fact, it is important to find a balance between the need to reduce the leukemia burden and avoiding excess toxicity. In the last years, hypomethylating agents such as azacitidine and decitabine have shown promising results in patients with MDS, particularly when clinical conditions do not allow the use of more conventional chemotherapy approaches (13, 14). Recently, these compounds have been associated with new agents like venetoclax, a selective inhibitor of BCL2, an anti-apoptotic protein that is over-expressed in some AML cells that are BCL2-dependent for survival (15). Although venetoclax alone has little direct activity on AML cells, synergic treatment with a hypomethylating agent can sensitize blast cells to the drug's pro-apoptotic activity (16). Recent data from treatment-naive, elderly AML patients with combined chemotherapy consisting of venetoclax and hypomethylating agents have shown promising outcomes (17). Little data is available on pediatric patients, and their use in patients with SDS is anecdotal. Here we describe the case of a boy affected by SDS-associated MDS/AML who was treated with conventional chemotherapy and then with venetoclax/azacitidine as a bridge therapy to HSCT.

Case presentation

We report the case of a 14-year-old boy affected by SDS that had been diagnosed in the first year of life with neutropenia, failure to thrive, and recurrent respiratory infections. In the following years, he had always been well, with moderate-to-severe neutropenia (400–600/mmc) but without severe infections. Yearly hematologic controls were performed, including regular bone marrow aspirates, with no evidence of myelodysplastic progression. When the patient was 16 years and 7 months old, he was admitted for persistent fever without other symptoms. Blood tests showed trilinear cytopenia: therefore, a bone marrow aspirate was performed, which showed a hypercellular marrow with the prevalence of erythroid precursors (50%), often with dysplastic signs, suggesting MDS. A bone marrow biopsy confirmed the presence of a hypercellular MDS with the prevalence of an immature erythroid population and an increase of megakaryocytes, which appeared dysplastic; flow cytometry analysis showed 8% of myeloid blasts (MDS with excess blasts —MDS EB1 according to WHO 2017). The recommendation to proceed to HSCT was formalized, and since he did not have compatible HLA siblings, a search for a matched unrelated donor (MUD) was started without starting any MDS-directed therapy. One month after the diagnosis, while waiting for the HSCT, he developed splenomegaly (2 cm below the left costal margin) and peripheral monocytosis. Repeated bone marrow aspirate showed progression to AML (bone marrow flow cytometry showed 75% blasts CD34 +/CD7+/CD45+/CD33+/CD11b dim/CD117+). Cytogenetic and molecular analysis (including RNA and DNA analysis for the main translocations associated with AML) showed no abnormalities. Treatment with Fludarabine—Cytarabine (FLA) was started (fludarabine 30 mg/m^2/day and cytarabine 2,000mg/m^2/day for 5 days), aiming to achieve leukemic burden reduction in view of HSCT. Treatment was well tolerated overall with no major organ toxicity, yet it was followed by an *Escherichia coli* sepsis requiring hospital admission (see **Figure 1** for the overall clinical course). Bone marrow re-evaluation showed morphologic remission (blasts 1%, confirmed by flow cytometry), with the persistence of abnormal hematopoiesis, compatible with pre-existent MDS. Maintenance therapy with low-dose i.v. cytarabine plus oral thioguanine was started, which was nevertheless discontinued due to worsening thrombocytopenia requiring platelet transfusions. Second hospital admission was required due to mucositis and febrile neutropenia, which responded to antibiotic therapy. Nevertheless, while arrangements for the MUD HSCT were being organized, AML relapsed (60% blasts in the bone marrow). Therefore a second FLA cycle was administered. This was again followed by neutropenic fever requiring hospitalization. After an initial improvement with antibiotic therapy, he developed persistent fever associated with marked hepatosplenomegaly and ascites [grade 2 according to Common Terminology Criteria for Adverse Events (CTCAE) v. 5.0]. Peripheral blood flow cytometry showed leukocytosis with 93% blasts, and bone marrow aspirates confirmed persistent massive leukemic infiltration

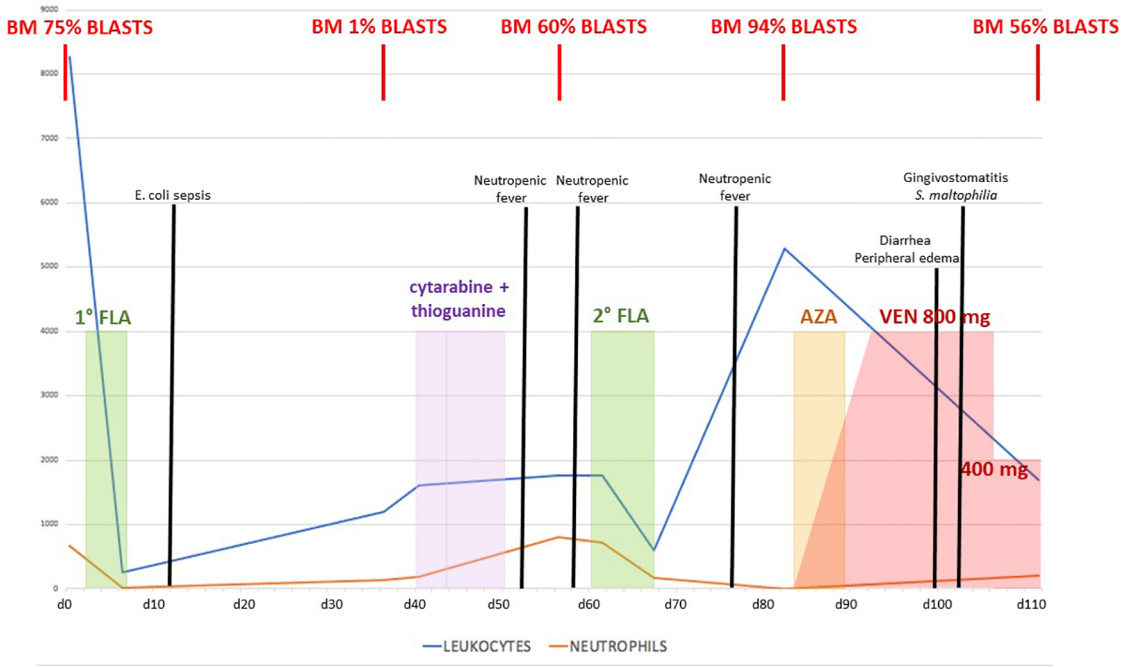

FIGURE 1
Graph presentation of chemotherapy treatment with blast count trend and clinical course complications.

(94% blasts). In consideration of the occurrence of severe toxicity from conventional chemotherapies, a second-line chemotherapy regimen was considered too dangerous. Therefore combination therapy with azacitidine (125 mg/day for 7 days) and venetoclax for 28 days was started, aiming to achieve disease control before proceeding to transplant. Venetoclax was started at a dose of 100 mg/day and gradually increased by 100 mg/day up to a dose of 800 mg/day. During the first 10 days of therapy, a gradual decrease of blasts in the peripheral blood was seen, with a marked decrease in hepatosplenomegaly. Therapy was overall well tolerated but was associated with diarrhea (grade 2 according to CTCAE v 5.0) and peripheral edema (grade 2 according to CTCAE v 5.0), a known side effect of venetoclax, as well as with an urticarial rash that resolved upon azacitidine discontinuation. Bone marrow examination performed at the end of the therapeutic course showed partial response on leukemic infiltration: the bone marrow appeared markedly hypocellular, thereby showing a definite response on AML burden in absolute terms (as compared to pre-treatment evaluation), even though leukemic blasts were still 52% by flow cytometry. Unfortunately, the patient developed a series of infectious complications following the end of the course, including severe *Stenotrophomonas maltophilia* gingivostomatitis (grade 3 according to CTCAE v 5.0), followed by two episodes of sepsis by *Staphylococcus epidermidis* and *Escherichia coli*, respectively. An increase of hepatosplenomegaly was also observed, likely due to AML progression, together with marked fluid overload only partially responding to diuretic therapy, and he was considered not eligible for HSCT or to continue therapy with a combination of azacitidine and venetoclax. Palliative therapy with azacitidine alone was started, with no response, and he eventually died from multiorgan failure.

Discussion

SDS is a rare and complex disease, and management of hematological manifestations can be challenging due to the absence of dedicated protocols and the intrinsic frailty of these patients. Often these patients undergo annual surveillance for MDS/AML with bone marrow, although the utility of this approach is controversial (18). When MDS occurs, nevertheless, the outcome is often poor. In the largest series of SDS patients with advanced MDS-AML, overall survival ranged from 10% for AML to 40% for MDS. In particular, mortality was due both to relapsed disease and to chemotherapy and transplantation-related toxicity, with almost half of patients dying before reaching HSCT (12–20). There is no consensus on the role of pre-HSCT cytoreductive chemotherapy, and data is sparse. When AML was diagnosed, we initially decided to use the FLA protocol due to the rapid progression of leukemia. Notably, AML in our patient showed a very good response to the first FLA course, achieving complete morphological and cytofluorimetric remission.

Unfortunately, as may be expected in a disease prone to accumulate new mutations, AML relapsed and became refractory to FLA chemotherapy. Although we did not perform a second cytogenetic analysis at relapse, it is possible that AML cells had acquired a complex karyotype, as described in refractory disease (21). Given worsening toxicity from chemotherapy courses, we chose to try a combination of venetoclax and azacitidine, based on adult experience and small pediatric case series (22–24). There is little data on experience with venetoclax for myeloid malignancies in children. The largest case series, reported by Winters et al., includes eight patients treated with venetoclax and azacitidine (two for MDS and six for AML), with morphologic response in six patients, including both patients with MDS and four AML patients. AML patients who responded did achieve negative minimal residual disease. Three patients eventually underwent HSCT. Therapy was well tolerated in all patients, and the most common adverse events were hematologic and gastrointestinal. One of the two patients with MDS was a 7-year-old girl with SDS who had developed MDS with excess blasts and monosomy 7. She was treated with venetoclax (at 800 mg/day-equivalent dose adjusted on body weight) and azacitidine 75 mg/m^2) with good response and reduction of bone marrow blast percentage to less than 1%. Notably, she underwent cord blood HSCT with primary graft failure and then proceeded to haploidentical HSCT with minimal toxicity after a conditioning regimen consisting of fludarabine, total body irradiation, anti-thymocyte globulin, and cyclophosphamide. In our patient, after the first 2 weeks of combination therapy, we witnessed a complete response in peripheral blast count and a marked reduction of hepatosplenomegaly. Bone marrow re-evaluation after the first month of therapy showed a partial response in relative terms (reduction of blasts from 92% to 52%) but with a marked reduction of AML burden, as the marrow appeared frankly hypocellular. Furthermore, although the response was not complete, the boy had received only one cycle of therapy: experience in adult patients treated with venetoclax/azacitidine for untreated AML showed a complete response after only one cycle in 40% of patients, reaching 66% after the second cycle with a median number of completed 28 days cycles of 7 (21, 25). It is possible to speculate therefore that another cycle could have further reduced the blast count prior to the HSCT. Notably, however, even though venetoclax/azacitidine has been shown to be well-tolerated even in frail patients, our patient developed several infectious complications and significant fluid retention, which are known toxicities of azacitidine/venetoclax therapy, eventually not allowing to proceed with HSCT. Future trials are needed to try to optimize venetoclax dosing strategies in frail patients, as reduced dosing might have been associated with better tolerance.

Overall, his clinical course was characterized by extreme instability and evanescence of response to therapies, with rapid relapse even after effective treatments. This was likely due to the absence of normal hematopoiesis in MDS arising in the context of the underlying SDS. In hindsight, considering the instability of the hematologic response in these patients, proceeding to HSCT at first disease remission (i.e., after the first FLA) could possibly have represented the best chance for this patient, albeit with all the limits and caveats associated with the need of using a haploidentical donor. Although haploidentical HSCT has been considered inferior to MUD for a long time, recent advances have led to similar outcomes, making this approach feasible for a wide type of diseases including AML patients (26). Specific data for SDS, nevertheless, are scarce. Cyclophosphamide, which is used for T-cell depletion *in vivo*, has been used in an adult patient with SDS and AML, suggesting that this method may be applicable also for patients with SDS, yet with some risk of cardiotoxicity (27). Another approach could be alpha/beta T-cell depletion, but experience in literature is limited for SDS, and cellular manipulation *ex vivo* was not available in our center.

Conclusion

Patients with SDS that develop MDS and AML are prone to high morbidity and mortality due to severe treatment-related toxicity and relapsed/refractory disease. With the limitations of observation of a single patient, our experience suggests that AML in SDS may respond to venetoclax/azacitidine combination therapy, which could therefore represent an option as a bridge to HSCT. Further studies are necessary to define the best treatment regimens and hopefully shared treatment recommendations for MDS/AML in these patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

SN and GZ designed the study. CI and VK collected and analyzed the data. AGG drafted the initial manuscript. NG, FV and MR revised the article critically. NM reviewed and edited the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case Report: Whole genome sequencing identifies *CCDC88C* as a novel *JAK2* fusion partner in pediatric T-cell acute lymphoblastic leukemia

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In the present report, we applied whole genome sequencing (WGS) to genetically characterize a case of pediatric T-cell acute lymphoblastic leukemia (ALL) refractory to standard therapy. WGS identified a novel *JAK2* fusion, with *CCDC88C* as a partner. *CCDC88C* encodes a protein part of the Wnt signaling pathway and has previously been described in hematological malignancies as fusion partner to *FLT3* and *PDGFRB*. The novel *CCDC88C*::*JAK2* fusion gene results in a fusion transcript, predicted to produce a hybrid protein, which retains the kinase domain of JAK2 and is expected to respond to JAK2 inhibitors. This report illustrates the potential of WGS in the diagnostic setting of ALL.

KEYWORDS
pediatric T-ALL, whole genome sequencing, JAK2 fusions, targeted therapy, precision medicine

Introduction

Current treatment protocols for pediatric acute lymphoblastic leukemia (ALL) use information regarding genomic aberrations in the leukemic blasts together with clinical features at presentation, and initial therapy response is used to assign patients to different risk categories and treatment intensities (1). Survival of pediatric acute ALL in risk-adapted chemotherapy trials exceeds 90% (2); however, further improvements in outcome are not likely to be achieved with conventional chemotherapy but will require alternative treatment approaches such as immunotherapy or targeted therapy.

High throughput sequencing technologies have shown enormous potential in the genetic characterization of hematological malignancies, particularly in pediatric ALL (3), and are paving the way to personalized treatment strategies, although implementation in clinical practice is still meager (4). Technologies that interrogate the whole genome with high resolution are increasingly being adopted in the

diagnostic workout of pediatric ALL (5). Here, we report a novel *JAK2* fusion in a pediatric case of therapy-refractory T-ALL that was detected using paired-end whole genome sequencing (WGS) in the diagnostic setting.

Case presentation

A 7-year-old boy presenting with a leukocyte count 800 × 10^9/L and peripheral lymphadenopathy was diagnosed with precursor T-ALL with extensive involvement of the bone marrow (BM) and peripheral blood. Multiparameter flow cytometry showed an immature T-lymphoblast population (95%) of cortical phenotype (EGIL III; CD45dim/CD7+/cytCD3+/Tdt+//CD4/CD8dim+/CD10−/CD1ahetero/CD56/16−/CD99++/CD5dim+/ CD2+/CD48+/CD38+//TCRa/b−/TCRg/d−/HLA−DR− {Bene, 1995 #41}). The blasts were negative for myeloid markers (cytMPO and CD33) and for B-cell markers (CD19 and cytCD79a). According to the standard of care (SoC) genomic characterization, the patient had a normal karyotype and none of the targeted fluorescence *in situ* hybridization (FISH) analysis was positive, i.e., no *KMT2A*- or *BCR::ABL* rearrangements and no *ABL*-class rearrangement were detected. A biallelic *CDKN2A/B* deletion was the only clonal aberration detected. The patient responded poorly to standard four-drug induction (dexamethasone, pegylated-asparaginase, daunorubicin, and vincristine) and was switched to consolidation at day 19 (6-mercaptopurine, cytarabine, and cyclophosphamide). Two weeks later, he still had 80% blasts in the bone marrow and was considered refractory and treatment with nelarabine initiated. Following two rounds of nelarabine and one additional high-risk chemotherapy block, remission was achieved, and he subsequently underwent hematopoietic stem cell transplantation (HSCT) from his haploidentical mother. The conditioning regimen consisted of ATG (grafalon), fludarabine, and thiotepa together with total body irradiation (TBI) (12 Gy in four fractions) and rituximab after which he received an alpha/beta T-cell-depleted peripheral stem cell graft. Initially, mycophenolate mofetil was switched to cyclosporin together with prednisolone after 45 days due to acute gut and skin graft versus host disease (GVHD). He is currently 8 months post HSCT, with no sign of disease but still on a low dose prednisolone.

At the time of initial failure, the genetic laboratory was engaged to screen for potential targets for experimental therapy. DNA isolated from the bone marrow sample taken at diagnosis was sequenced using a PCR-free, paired-end WGS protocol with a 30× coverage on an Illumina HiSeq X platform at Clinical Genomics (SciLifeLab, Stockholm) and annotated to the Human GRCh37 (hg19) build. Variants were identified using the MIP (6) validated for routine diagnostics and visualized in the SCOUT interface (7). MIP performs structural variant (SV) detection using Delly (8), TIDDIT V2.0 (9), as well as Manta (10) and single nucleotide variant (SNV) detection using GATK HaplotypeCaller (11). Subsequently, filtering was performed using the list of targetable genes described in the INFORM study (12). This analysis resulted in 260 SNVs and 18 SVs. Further filtering of the SNVs based on gnomAD or local observations as well as annotations in ClinVar narrowed the list to 11 SNVs that were manually inspected and dismissed, as no targetable SNV was identified. Similarly, the 11 SVs were manually curated based on recurrency in the local database and inspection in

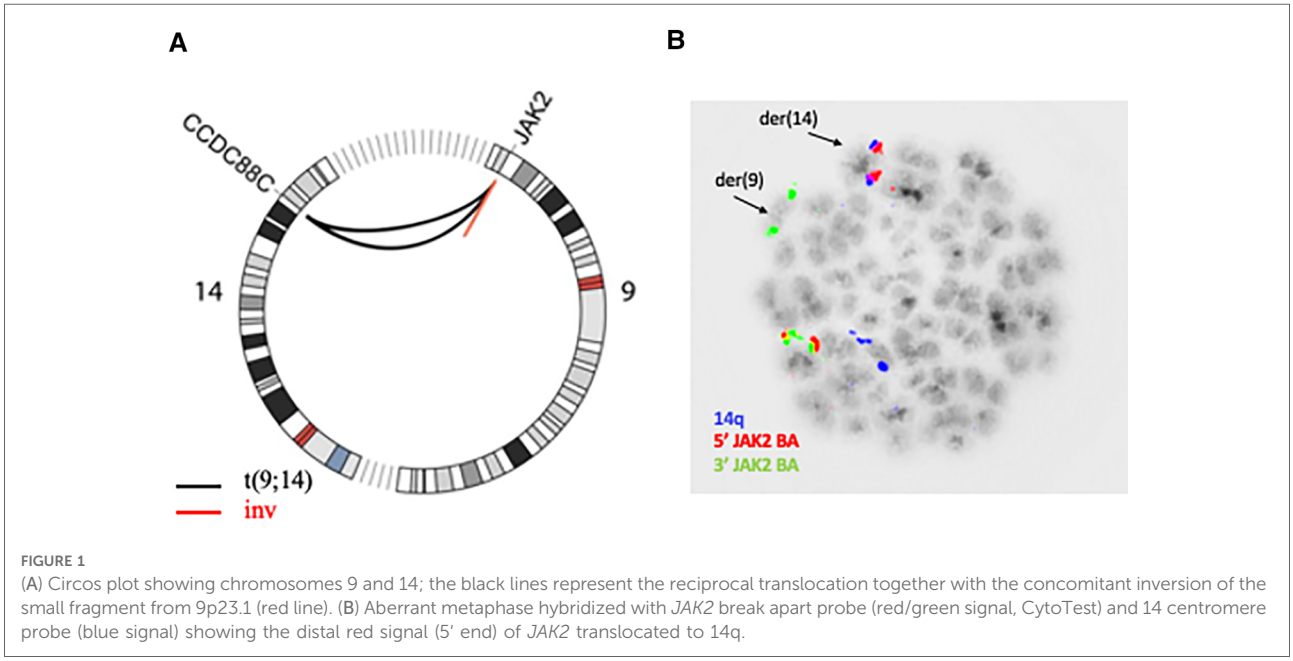

FIGURE 1
(A) Circos plot showing chromosomes 9 and 14; the black lines represent the reciprocal translocation together with the concomitant inversion of the small fragment from 9p23.1 (red line). (B) Aberrant metaphase hybridized with *JAK2* break apart probe (red/green signal, CytoTest) and 14 centromere probe (blue signal) showing the distal red signal (5′ end) of *JAK2* translocated to 14q.

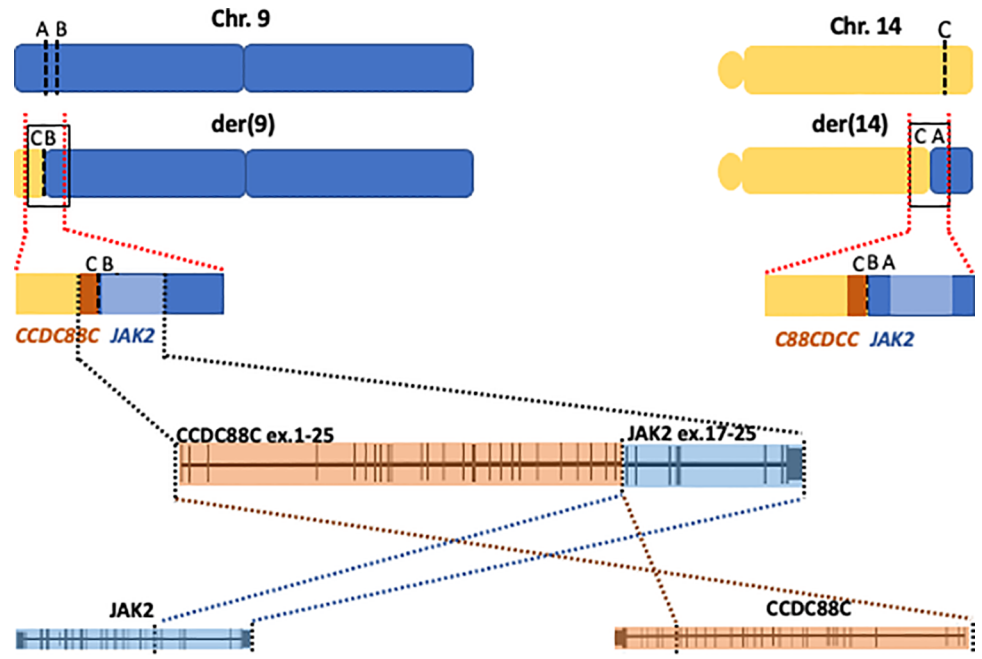

FIGURE 2
Cartoon showing a schematic representation of chromosomes 9 (blue) and 14 (yellow) at the derivative chromosomes 9 and 14 (upper panel), the black squares mark the junctions. Middle cartoon represents a blow up of the junctions illustrating the creation of the fusion gene on derivative chromosome 9 (der9). The lower panel represents a magnification of the fusion gene showing exons 1–25 in *CCDC88C* joined to exons 16–25 in *JAK2*.

Integrated Genomic Viewer (IGV) (13) leaving only three SVs that interestingly affected regions recurrently involved in ALL.

Two of these SVs affected the *JAK2* locus on 9p24.1 and inspection in IGV revealed a shared breakpoint suggesting that both SVs had occurred in the same genomic rearrangement (**Supplementary Figure 1**). The first SV consisted in a 122 kb-long intrachromosomal inversion with breakpoints that mapped to intron 15 (NM_001322194.2) and telomeric to the *JAK2* locus. The second SV consisted in an interchromosomal event and shared the breakpoint in intron 15 of *JAK2*, while the second breakpoint was located at 14q32.11 and mapped to intron 25 in the *CCDC88C* locus (NM_001080414). Both events are visualized in the Circos plot in **Figure 1A**. The rearrangement of *JAK2* could be verified with metaphase FISH that confirmed that the 5′ signal at the JAK2 locus was translocated to chromosome 14 while the 3′ signal was retained on derivative chromosome 9 (**Figure 1B**). As the rearrangement involves the most distal regions of chromosomal arms 9p and 14q, it is beyond the resolution of chromosome banding analysis and cannot be detected by karyotype analysis. In addition, SV analysis also detected a deletion on 9p21.3 encompassing 39 kb and supported by 95% of reads that resulted in a biallelic loss of *CDKN2A/B* that had been previously found in SoC testing.

Taken together the data indicated a reciprocal translocation t(9;14)(p24.1;q32.11) with a concomitant inversion of a 122-kb-long fragment from 9p that resulted in the creation of a fusion gene joining intron 25 of *CCDC88C* to intron 15 of *JAK2* on derivative chromosome 9. As a result of the inversion from the 9p fragment, the genes are arranged in opposite orientations and thus no reciprocal fusion is created at derivative 14 (**Figure 2**).

According to the WGS findings, the rearrangement juxtaposes exons 1 to 25 of *CCDC88C* to exons 16 through 25 of *JAK2*, creating a novel fusion gene (**Figure 2**). The expression of the fusion transcript joining exon 25 of *CCDC88C* (3′ partner) to exon 16 in *JAK2* gene (5′ partner) was confirmed with RT-PCR followed by Sanger sequencing. The fusion gene will thus result in an in-frame hybrid protein with the kinase domain of JAK2 at the carboxy-terminal.

Discussion

JAK2 encodes a nonreceptor tyrosine kinase involved in cell proliferation and differentiation through the JAK/STAT signaling pathway. Aberrant *JAK2* is found in several cancers including both myeloid and lymphoid malignancies (14). Fusions involving *JAK2* were found in Philadelphia-like ALL (15), a subset of high-risk B-ALL characterized by a distinctive gene expression profile resembling Philadelphia-positive ALL, but lacking the canonical *BCR::ABL* fusion (16,

17). A fraction of Philadelphia-like ALL harbors *JAK2* fusions where *JAK2* is the 5′ partner and all the fusions described so far include exons 19–25 and retain the kinase domain that is constitutively activated in the fusions (18). To this date, over 30 genes have been found as potential 3′ partners to *JAK2*, but this is the first report of a *CCDC88C*::*JAK2* fusion. JAK2 inhibitors have shown antileukemic effect in *ex vivo* experiments in cells carrying *JAK2* fusions (19, 20), that include the kinase domain of JAK2; however, the experience *in vivo* is limited (21). *JAK2* fusions have only been described in isolated cases of T-ALL (18), although activating point mutations in members of the JAK/STAT pathway has been reported in up to 25% of patients with T-ALL (22). Patients carrying genomic alterations that activate the JAK/STAT pathway either through gain of function mutations or gene fusion that include the JAK2 kinase domain are potential candidates for targeted therapy (23) (24). Pediatric patients with ALL and genomic features consistent with JAK/STAT pathway activation are currently recruited in ongoing trials (clinicaltrials.gov NCT03117751 and NCT02723994) for targeted treatment with ruxolitinib (selective JAK1/2 inhibitor) in addition to chemotherapy. Since our patient responded to nelarabine, he was never considered for therapy with JAK inhibitors nor were cells available to perform *in vitro* sensitivity tests.

CCDC88C is a ubiquitous protein, involved in the Wnt signaling pathway, with multiple functions that include determination of cell polarity, development of the nervous system, and tumor suppressor activity (25). CCDC88C protein is highly expressed in B- and T-cell pediatric leukemias (https://pecan.stjude.cloud/proteinpaint/CCDC88C). *CCDC88C* has been reported a handful occasions in hematological malignancies as fusion partner with *FLT3* (26) as well as *PDGFRB* (27) including a case report of pediatric patient with a myeloproliferative disorder (28). It has also been reported as the 5′ fusion partner to PDGFRB in a young adult with Philadelphia-like ALL that responded to imatinib (29).

The genetic landscape of T-ALL is widely heterogeneous, and despite several recurrent aberrations, to date no single lesion is used to risk-stratify T-ALL patients. However, certain aberrations such as BCR-ABL and other ABL-class fusions or KMT2A rearrangements, although rare, contribute important information to individualized patient management.

Since our patient responded to nelarabine, he could be bridged to transplantation and experimental therapy with JAK2 inhibitors was not considered. However, this report illustrates the power of WGS in the diagnostic setting of ALL and describes a novel *CCDC88C*::*JAK2* fusion in T-ALL, adding *CCDC88C* to the list of potential *JAK2* partners. While the validation of WGS in the diagnostic setting of acute leukemias is still ongoing in our laboratory, the present report serves as a real-life example of the power of WGS in the diagnostic setting of acute leukemia, as it enables timely recognition of potential therapeutic targets in high-risk pediatric ALL.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was approved by the Ethical Review Board at Stockholm County. Written informed consent was obtained from the patient's legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article. The study was conducted in accordance with the World Medical Association's Declaration of Helsinki.

Author contributions

AK, LS and AH performed data acquisition, AK, FR, and GB interpreted the data and wrote the manuscript. PS provided patient care and contributed to writing the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Case report: Tisagenlecleucel for treatment of relapsed B- acute lymphoblastic leukemia in a patient with *CHEK2* mutation

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Background: Germline Checkpoint Kinase 2 gene (*CHEK2)* mutations can increase the risk of solid tumors. Recently, they have been identified as risk factors for hematologic malignancies. However, to the best of our knowledge, B-acute lymphoblastic leukemia (B-ALL) has never been described as a presenting manifestation of germline *CHEK2* mutation. Chimeric antigen receptor-T (CAR-T) cell therapy directed against CD19 antigen (tisagenlecleucel) is a novel cellular therapy for treatment of relapsed/refractory (R/R) B-ALL. The use of tisagenlecleucel has not been described in patients with *CHEK2* mutation.
Case Presentation: We describe a case of a pediatric patient with a heterozygous pathogenic germline *CHEK2* mutation (c.1100delC; p.Thr367Metfs*15) successfully treated with tisagenlecleucel for relapsed B-ALL to avoid hematopoietic cell transplant (HCT). The twelve-year-old boy was diagnosed with National Cancer Institute (NCI) high-risk B-ALL (white blood cell count >50,000/mcL), with no extramedullary disease. Cytogenetic analysis revealed normal karyotype but fluorescent *in situ* hybridization (FISH) showed 93% positivity for *CRLF2::P2RY8* rearrangement. He was treated as per Children's Oncology Group (COG) AALL1131 therapy and achieved a complete remission. Seven months after diagnosis, he was found to have papillary thyroid carcinoma with no evidence of metastatic disease. The patient underwent a total thyroidectomy with central lymph node biopsy and radioactive iodine therapy. The patient's biological mother and fraternal twin brother carry the same germline *CHEK2* mutation with no history of malignancy. The biological father tested negative for the familial mutation. The patient's genetic panel also identified three variants of unclear significance: *CDKN2A* (c.37 °C > T; p.Arg124Cys), *FLCN* (c.62G > A; p.Cys21Tyr) and *SDHAF2* (c.139A > G; p.Met47Val). Extended family history also revealed a diagnosis of anaplastic thyroid cancer in maternal uncle at the age of 44 years. Fifteen months after diagnosis the patient had a relapse of B-ALL (both medullary and extramedullary with blasts in CSF), which was successfully treated with tisagenlecleucel. The patient remains in remission 3 years after receiving tisagenlecleucel.
Conclusion: As conventional chemotherapy and radiation can potentially increase the risk of DNA damage and development of secondary malignancies, CD19 CAR-T therapy (tisagenlecleucel) can be used as a substitute for intensive re-induction chemotherapy and HCT in patients with a germline *CHEK2* mutation.

KEYWORDS

CAR-T, tisagenlecleucel, B-ALL, CHEK2 mutation, MDS

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Introduction

Checkpoint kinase 2 (*CHEK2*) is a tumor suppressor gene that plays a crucial role in the cell cycle and response to DNA damage induced by replication stress and double stranded DNA breaks. CHK2 kinase, a protein coded by the *CHEK2* gene, is also required during mitosis for spindle formation, accurate attachment of kinetochores, and subsequent proper chromosome segregation. Therefore, *CHEK2* mutations can lead to aberrant proteins, contributing to errors in chromosome segregation and a higher frequency of unbalanced structural rearrangements (1). Whether the *CHEK2* gene is a true cancer predisposition syndrome gene by itself remains a topic of debate, but emerging evidence suggests an important role in cancer susceptibility, especially in relation to breast cancers (2, 3). Truncating *CHEK2* gene mutations are well-known pathogenic variants, but the clinical significance of missense variants are subject of ongoing research and their interpretation remains challenging (4). Heterozygous pathogenic germline mutations in *CHEK2* (c.1100delC) have been associated with an increased risk of hereditary breast, prostate, kidney, thyroid, and colon cancers (5–7). Congenital *CHEK2* inactivation is also associated with an increased risk of hematologic malignancies, mainly myeloid neoplasms like myelodysplastic syndrome (MDS) (8). A recent study showed a two times higher frequency of unbalanced structural chromosomal rearrangements (58%) among patients harboring germline *CHEK2* mutations, in comparison to non-carriers (27%) in MDS patients (9). However, lymphoid malignancies, especially B-ALL, seems to be a very rare occurrence in association with *CHEK2* mutations. To the best of our knowledge, there has been no report on the use of tisagenlecleucel for relapsed B-ALL in a patient with a *CHEK2* mutation (c.1100delC). Herein, we report the successful use of tisagenlecleucel for the treatment of early relapsed (both medullary and extramedullary) pediatric B-ALL in a patient with a *CHEK2* mutation and papillary thyroid carcinoma.

Case presentation

A 12-year-old boy with a history of hypothyroidism presented with generalized petechiae and hematuria. The patient's white blood count at presentation was 446,750/microliter with mild anemia (hemoglobin 10.6 g/dl), severe thrombocytopenia (24,000/microliter) and 90% B-lymphoblasts in peripheral blood. Lumbar puncture (LP) revealed no blasts in cerebrospinal fluid (CSF). Cytogenetic analysis of bone marrow (BM) blasts revealed a normal karyotype, but fluorescent *in situ* hybridization (FISH) analysis revealed a 93% positivity for *CRLF2::P2RY8* rearrangement. The patient subsequently started therapy as per Children's Oncology Group AALL1131 chemotherapeutic regimen. Family history revealed a diagnosis of metastatic thyroid cancer in a maternal uncle at the age of 44 years who died at the age of 45 years (**Figure 1**).

Seven months after the diagnosis of B-ALL, he presented with a thyroid mass. Fine needle aspiration cytology was diagnostic of papillary thyroid cancer, staged at Bethesda Category V (**Figure 2A**). Evaluation for metastatic disease was negative. The patient underwent a total thyroidectomy with central lymph node biopsy followed by radioactive iodine therapy. He was referred to Genetics due to concerns for a cancer predisposition syndrome given the development of multiple malignancies within a year. Genetic testing revealed a pathogenic heterozygous *CHEK2* gene mutation (c.1100delC; p.Thr367Metfs*15). The genetic panel also identified three variants of unclear significance: *CDKN2A* (c.37 °C > T;p. Arg124Cys), *FLCN* (c.62G > A; p.Cys21Tyr) and *SDHAF2* (c.139A > G; p.Met47Val). A whole-body MRI was negative for any other malignancies.

Fifteen months after his initial diagnosis, while on maintenance chemotherapy, the patient developed a frontotemporal headache. Computed tomography (CT) scan of the brain showed no intracranial involvement. Mild swelling of optic nerve head was noted on ophthalmologic examination. Increased intracranial pressure and central nervous system (CNS) disease was confirmed by lumbar puncture and magnetic resonance imaging of the brain.

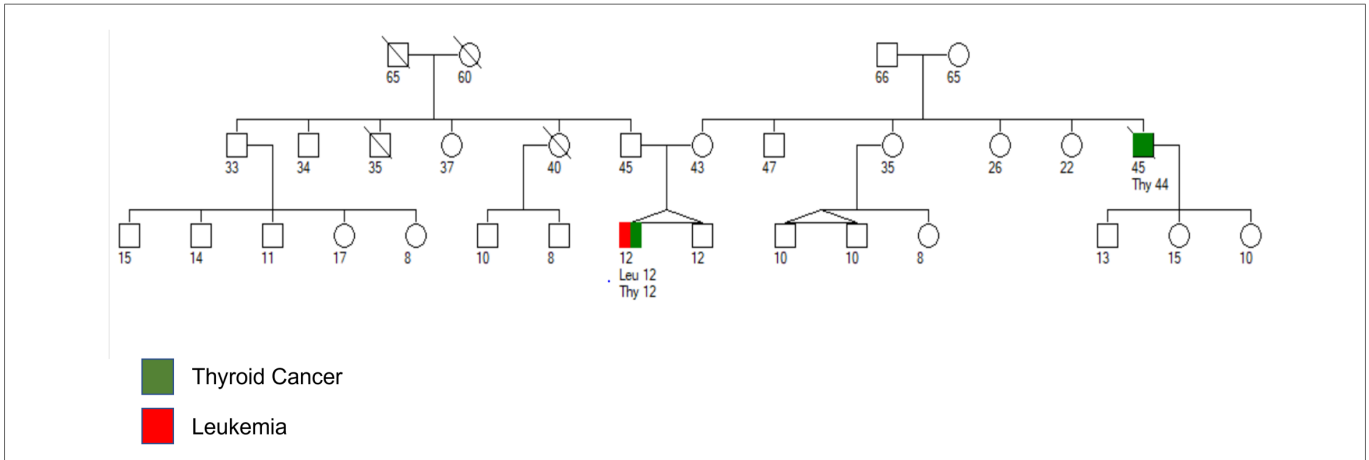

FIGURE 1
Pedigree chart with age of the patient and family members in years. Maternal uncle was diagnosed with anaplastic thyroid cancer at the age of 44 years and died from metastatic disease at the age of 45 years. Patient was diagnosed with papillary thyroid carcinoma and B-ALL at the age of 12 years.

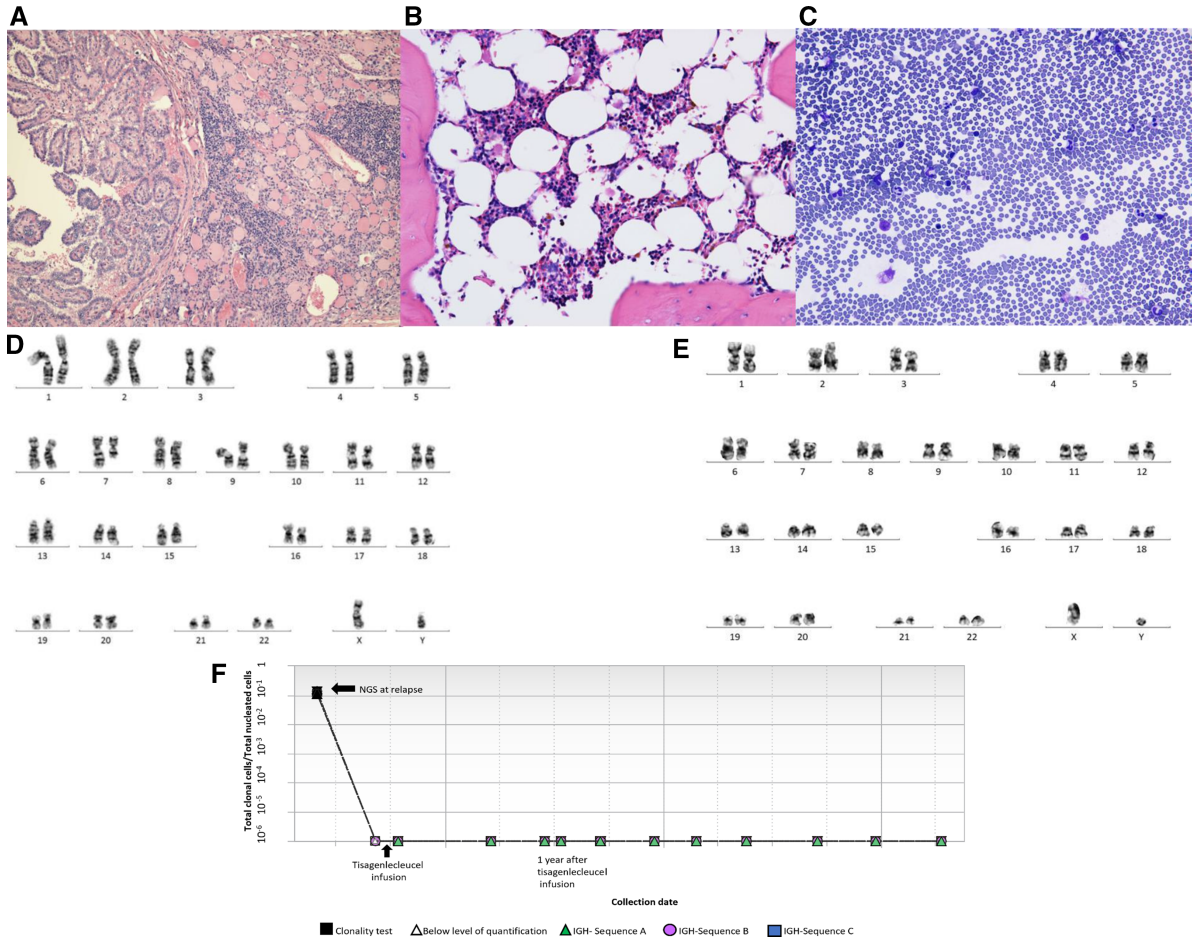

FIGURE 2
(A) Thyroidectomy reveals thyroid tissue with focal chronic inflammation and a papillary carcinoma (H&E stain, 100×). (B) Representative bone marrow biopsy reveals hypocellular marrow with trilineage hematopoiesis and no overt dysplasia (H&E stain, 400×). (C) Representative bone marrow aspirate reveals few erythroid and granulocytic cells with no overt dysplasia (Giemsa stain, 400×). (D) Karyotyping showing deletion of 7q. (E) Karyotyping showing absence of deletion 7q in most recent bone marrow specimen. (F Next generation sequencing (NGS) showing detection of leukemic clones at relapse and remission (0 residual clonal cells) at 1, 2 and 3 years after receiving tisagenlecleucel.

Cerebrospinal fluid studies revealed elevated WBC (48/microliter) with 75% lymphoid blasts. Bone marrow aspirate revealed 15% lymphoid blasts by flow cytometry with FISH positive for *CRLF2::P2RY8* rearrangement suggesting relapse with the same leukemic clone that was found on initial diagnosis. Both BM and CSF lymphoid blasts were positive for CD19. He had no signs of any other extramedullary site involvement. He was started on bi-weekly triple intrathecal chemotherapy, in addition to systemic salvage chemotherapy consisting of vincristine, dexamethasone, and mitoxantrone for the next several weeks. The patient was eventually negative for CNS disease sixteen months after initial diagnosis.

In preparation for CAR-T therapy the patient underwent apheresis. The patient was then initiated on a bridging therapy with non-escalating Capizzi protocol prior to receiving tisagenlecleucel. Pre-CAR-T infusion BM and CSF evaluation (done prior to starting lymphodepletion) showed no blasts by flow cytometry. He subsequently received lymphocyte-depleting chemotherapy with fludarabine and cyclophosphamide prior to his infusion of tisagenlecleucel. Four days after CAR-T infusion, the patient developed grade 2 cytokine release syndrome (CRS) but did not develop any immune-effector cell associated neurotoxicity syndrome as per American Society for Transplantation and Cell Therapy Consensus Grading. The patient received tocilizumab and a short course anakinra (3 days). A CSF study was done on day 5 for severe headache in the setting of high-grade fever not responding to tocilizumab, which revealed 70 WBCs/uL with 72% lymphocytes (7% CD19 positive lymphoblasts along with atypical lymphocytes). Day 30 BM evaluation was negative for minimal residual disease by flow cytometry and confirmed by next generation sequencing (NGS) by clonoSEQ (Adaptive). CSF studies at the time showed increased lymphocytes but no blasts. Follow up LPs and BM aspirate/biopsies since then have been consistently negative for lymphoid blasts by flow cytometry. Of note, chromosomal analysis by karyotyping of the patient's bone marrow aspirate, taken 15 months post CAR-T, showed two abnormal clones in metaphase cells. The first clone revealed that 17% of cells had an unbalanced rearrangement of chromosome 7q, which

resulted in a partial deletion of 7q, an anomaly frequently associated with primary and secondary myeloid disorders, including MDS. However, no morphologic evidence of myelodysplasia was noted on BM biopsy (**Figures 2B,C**). The second clone revealed that 13% of cells had a balanced reciprocal translocation (1;19) (q23;p13.3), which results in a TCF3-PBX1 fusion. However, concurrent interphase FISH studies were negative for this fusion. Serial BM biopsies and cytogenetic testing continues to reveal intermittent partial deletion of the distal portion of 7q (**Figures 2D,E**), while the findings of translocation (1;19)(q23;p13.3) have resolved. His B-ALL remains in remission with no abnormal clones identified on serial NGS testing (**Figure 2F**) or by immunophenotyping (flow cytometry) and has persistent B cell aplasia. Due to prior history of intensive chemotherapy, germline *CHEK2* mutation and deletion 7q, we suspected a diagnosis of MDS but the follow up CBCs and BM examinations did not confirm our diagnosis due to following reasons. Firstly, the patient's complete blood count improved during the follow up and did not show any progression of cytopenia. Secondly, the chromosomal abnormality of deletion 7q was detected intermittently at a very low percentage that was only detected by karyotyping but not by FISH. Finally, this abnormality was not detected on the patient's most recent bone marrow examination (55 months after initial diagnosis of B-ALL), both on FISH and cytogenetics. We continue to follow this patient closely with serial (every 3- 6 month) CBCs and bone marrow examinations. A summary of the patient's clinical course can be found in **Figure 3**.

Discussion

Checkpoint kinase 2 is a tumor suppressor gene that plays a crucial role in the cell cycle and response to DNA damage through the ATM-CHEK2-p53 DNA damage response pathway (1). While truncating loss of function (LoF) mutations are known to be pathogenic, several variants of unknown significance have been described with undetermined functional and clinical significance. Homozygous LoF *CHEK2* mutations can present as Li-Fraumeni (LFS)-like syndrome, whereas heterozygous LoF *CHEK2* variants are moderate penetrance risk factors for solid tumors (2–7). Not surprisingly, emerging evidence also supports the association of certain *CHEK2* gene mutations with myeloid and lymphoid malignancies. LoF *CHEK2* mutations are now increasingly recognized as risk factors for myeloid malignancies (myeloproliferative neoplasms, myelodysplastic syndromes, and acute myeloid leukemia) (8, 9). Interestingly, pre-clinical murine models suggest a role of *CHEK2* mutations (*CHEK2* c.1100delC allele) in development of hematopoietic malignancies besides other solid tumors like breast and lung cancer (10). This is supported by clinical findings in large scale epidemiological studies of these malignant neoplasms (5–7). It must be noted that our patient had other cytogenetic abnormalities and molecular mutations besides *CHEK2* mutation that most likely led to the development of B-ALL, and it is unlikely that the *CHEK2* mutation was the primary driver mutation. However, given the evidence supporting association of *CHEK2* mutation with MDS, the karyotypic findings of clones harboring MDS defining cytogenetic findings (partial deletion of the distal portion of 7q) is concerning for an increased risk of MDS in the future. Therefore, the patient is undergoing close observation with yearly bone marrow examination besides screening for other solid tumors.

The finding of germline *CHEK2* mutation in a patient with relapsed/refractory hematological malignancy has profound clinical implications and presents several challenges. Choosing matched sibling donor (MSD) with the same germline *CHEK2* mutation may present as a clinical dilemma, even though there is no definitive evidence to suggest relapse of leukemia or graft failure after MSD HCT. In our patient we avoided HCT due to availability of CAR-T therapy, the possible need for additional chemotherapy for *CHEK2* mutation-associated solid tumors in the future, and the risk of therapy-related MDS or other secondary malignancies. The use of tisagenlecleucel has been previously described in a patient with LFS and B-ALL, but it has not been described in patients with *CHEK2* mutations (11). Also, the patient did not have a well-matched unrelated donor or umbilical cord blood units. The patient's mother and fraternal twin brother also harbored the same *CHEK2* gene mutation, though the father did not. However, father was not available as a potential donor. Therefore, haploidentical HCT was not considered a viable option. Some reports suggest that relapsed/refractory pediatric B-ALL treated with HCT after CAR-T infusion has significantly better median overall survival than the non-transplant group, especially if post CAR-T NGS is positive even at low levels (12, 13). However, the utility of HCT for patients in deep remission showing no

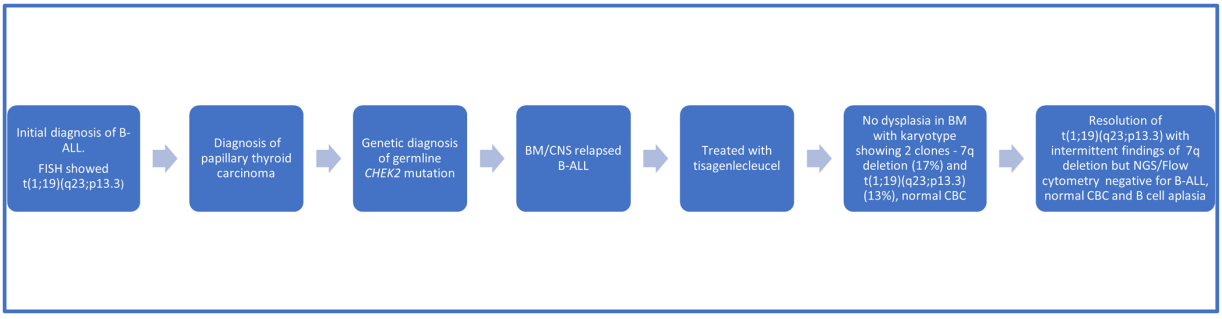

FIGURE 3
Timeline of clinical events.

(zero) leukemic clones remains uncertain and could probably be observed without consolidation with HCT (13). Therefore, in our patient with NGS negative remission, we have taken the approach of close monitoring with NGS testing without pursuing HCT. In the future, novel agents like poly (ADP-ribose) polymerase (PARP) inhibitors may be a potential therapeutic option for patients with *ATM* or *CHEK2* mutation associated malignancies (8). The patient's fraternal twin and mother have also undergone genetic counseling and been educated regarding the need for cancer surveillance.

In summary, we describe the case of a pediatric patient with a heterozygous *CHEK2* mutation (c.1100delC (p. Thr367Metfs*15) who presented with high-risk B-ALL and papillary thyroid cancer at the age of 12years. The patient's early relapsed B-ALL with bone marrow and CNS involvement was successfully treated with tisagenlecleucel. The patient responded and tolerated tisagenlecleucel very well. He continues to be in NGS-negative remission 3 years after treatment. The significance of intermittent findings of deletion of 7q on serial BM examinations in the absence of cytopenia and morphologic evidence of dysplasia remains a diagnostic dilemma and does not fulfill the criteria for diagnosis of MDS. However, it is concerning for potential development of either *CHEK2* mutation-associated or therapy-related MDS in future. Hence, we continue to follow this patient closely with serial (every 3- 6 month) CBCs and bone marrow examinations. Moreover, the implication of infused *CHEK2* mutations harbored by autologous CAR-T cells in the long term remains unknown. Our case highlights the need for more research in delineating the true role of pathogenic *CHEK2* gene mutations in hematologic malignancies as a potential cancer predisposition gene. Finally, our case also illustrates that CAR-T therapy can be considered for treatment of R/R B-ALL patients with potential cancer predisposition syndromes such as *CHEK2* mutation, in order to avoid HCT as has been previously described for a patient with LFS who developed B-ALL (11).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the study was exempt from ethical approval procedures. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

AI and AV: were involved in drafting, review, and revision of the initial and final manuscript. AA and DJ: were involved in the patient's treatment decision as well as the review and revision of the initial manuscript. JT and MB: were involved in genetic consultation as well as providing the pedigree chart, review, and revision of the initial manuscript. JC: performed pathologic examination of the bone marrow slides and provided pictures of bone marrow aspirate patient's karyotyping, next generation sequencing studies as well as the review and revision of the initial manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Immune dysregulation in Kabuki syndrome: a case report of Evans syndrome and hypogammaglobulinemia

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Kabuki syndrome (KS) is a rare multisystemic disease due to mutations in the *KMT2D* or *KDM6A* genes, which act as epigenetic modulators of different processes, including immune response. The syndrome is characterized by anomalies in multiple organ systems, and it is associated with autoimmune and inflammatory disorders, and an underlying immunological phenotype characterized by immunodeficiency and immune dysregulation. Up to 17% of KS patients present with immune thrombocytopenia characterized by a severe, chronic or relapsing course, and often associated to other hematological autoimmune diseases including autoimmune hemolytic anemia, eventually resulting in Evans syndrome (ES). A 23-year-old woman, clinically diagnosed with KS and presenting from the age of 3 years with ES was referred to the Rare Diseases Centre of our Pediatric Department for corticosteroid-induced hyperglycemia. Several ES relapses and recurrent respiratory infections in the previous years were reported. Severe hypogammaglobulinemia, splenomegaly and signs of chronic lung inflammation were diagnosed only at the time of our observation. Supportive treatment with amoxicillin-clavulanate prophylaxis and recombinant human hyaluronidase-facilitated subcutaneous immunoglobulin replacement were immediately started. In KS patients, the failure of B-cell development and the lack of autoreactive immune cells suppression can lead to immunodeficiency and autoimmunity that may be undiagnosed for a long time. Our patient's case is paradigmatic since she presented with preventable morbidity and severe lung disease years after disease onset. This case emphasizes the importance of suspecting immune dysregulation in KS. Pathogenesis and immunological complications of KS are discussed. Moreover, the need to perform immunologic evaluations is highlighted both at the time of KS diagnosis and during disease follow-up, in order to allow proper treatment while intercepting avoidable morbidity in these patients.

KEYWORDS

Kabuki syndrome, Evans syndrome, autoimmunity, immunodeficiency, hypogammaglobulinemia, immune dysregulation

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Introduction

Kabuki syndrome (KS) is a rare, multiple congenital anomaly/intellectual disability syndrome with an estimated prevalence of 1:32,000 in Japan (1), where it was first described in 1981. The prevalence outside Japan is presumably similar to that seen in the Japanese population, but is not known (2). In 2010, Ng et al. identified heterozygous mutations in *KMT2D* as the main genetic cause of KS (3). More recently, mutations in *KDM6A* have been reported in almost 5% of KS cases. However, genetic basis of the syndrome is still unknown in up to 20%–25% of the patients (4). Both *KMT2D*, inherited as autosomal dominant, and *KDM6A*, inherited as X-linked, are involved in embryogenesis, development and immune response, functioning as epigenetic modulators explaining the characteristic phenotype of KS patients (5, 6).

Immune abnormalities may occur later in childhood, being characterized by immune dysregulation with increased risk of autoimmune diseases and immunodeficiency and, therefore, of infections (7). Among autoimmune diseases, immune thrombocytopenic purpura (ITP), hemolytic anemia [often combined in Evans syndrome (ES)], thyroiditis and vitiligo are reported (8–10). Autoimmune cytopenia associated with KS usually present with a chronic and relapsing course and a poor response to conventional therapy (11–14). Non-malignant lymphoproliferation has also been described as a feature of KS, similarly to other inborn errors of immunity (15, 16).

We report the case of a 23-year-old woman with KS and a history of several relapses of ES, referred to the Rare Diseases Centre of our Pediatric Department for corticosteroid-induced hyperglycemia. Severe hypogammaglobulinemia was also diagnosed at the time of our first observation.

Case description

A 23-year-old woman, clinically diagnosed with KS at the age of 5 years, was referred to our Rare Diseases Centre for hyperglycemia following high-dose prednisone treatment due to an ES relapse. The patient was born at term from cesarean section due to fetal distress. She was the third daughter of healthy Italian non-consanguineous parents, with no family history of immunological, autoimmune or rare diseases. Neonatal examination highlighted bifid uvula. At the age of three, she was diagnosed with autoimmune hemolytic anemia (AIHA) with a positive direct antiglobulin test, needing long-term corticosteroid therapy. During infancy she also presented with several episodes of sinusitis, otitis media and three episodes of pneumonia that required hospitalization. Moreover, she was diagnosed with ostium secundum atrial septal defect, surgically corrected at the age of 5 years. During hospitalization, genetic counseling highlighted the presence of major criteria for clinical diagnosis of KS including moderate intellectual disability, postnatal short stature, skeletal abnormalities, dermatoglyphic anomalies and facial dysmorphism (**Figure 1**).

Shortly afterward, the patient presented with ITP which, associated with AIHA, led to ES diagnosis. Over the years, the patient underwent several courses of corticosteroids due to ES relapses; however, since the age of 12, she had been poorly followed in any aspect of her syndrome except for neuromuscular rehabilitation.

At the time of our observation, at the age of 23 years, the patient presented with a peculiar KS facial appearance, short stature, obesity (BMI 43 kg/m^2), mild developmental delay, multiple skeletal defects (dorsal kyphosis, lumbar hyperlordosis, bilateral pronated feet) and dermatoglyphic anomalies. Moreover, she presented with clinical side effects of systemic glucocorticoids therapy including *Striae rubrae*, central obesity, and arterial hypertension. Severe hyperglycemia (glucose level >400 mg/dl) with elevated HbA1C level (>9%) led to glucocorticoid-induced diabetes mellitus diagnosis. GAD65, ICA and IA-2A autoantibodies were not detected. Serum glucose concentration had always been lower than 160 mg/dl before prednisone treatment.

Short-term glycemic control with a target range of 100–180 mg/dl throughout the day was chosen. Routine blood glucose

FIGURE 1
Patient's facial features (arched and broad eyebrows, long palpebral fissures, eversion of the lower eyelid, depressed nasal tip and short columella) and persistence of fetal fingertip pads, typical of Kabuki syndrome.

monitoring revealed a typical pattern of steroid-induced diabetes characterized by near-normal fasting glucose levels followed by hyperglycemia during the day. Standard dietary counseling and short-acting insulin were therefore initiated.

Further investigations indicated mild bilateral sensorineural hearing loss, renal anomalies and alternating strabismus. Echocardiography documented bilateral ventricular wall thickening, first-degree diastolic dysfunction of the left ventricle, and right ventricular systolic function below normal range. Abdominal ultrasound showed hepatic steatosis, mild splenomegaly (spleen longitudinal diameter = 12.6 cm), and bicornuate uterus. High-resolution computed tomography (HRCT) of the lungs revealed diffuse ground-glass opacities, interlobular septal and bronchial wall thickening, subsolid nodules with blurred edges, peribronchial calcifications and low-attenuation areas due to air trapping (**Figure 2A**). These findings were interpreted as interstitial lung disease and chronic inflammation, likely due to both recurrent respiratory infections and immune dysregulation, while monoclonal lymphoproliferative disorders were excluded. Given the diagnosis of ES, the chronic lung disease and the splenomegaly, an immunological evaluation was performed highlighting severe hypogammaglobulinemia: IgG 200 mg/dl (reference value 700–1,600); IgA 2 mg/dl (reference value 68–400) and IgM 149 mg/dl (reference value 40–259). T-cell phenotype showed: $CD3^+$ = 574 cells/μl ($CD4^+$ = 298 cells/μl, 29%; $CD8^+$ = 219 cells/μl, 21%). B- cell phenotype was characterized by a normal circulating B-cell number ($CD19^+$ B-cells = 364 cells/μl), with a reduction in IgD^+CD27^+ memory (3%) and IgD^-CD27^+ switched memory (2.5%) B-cells percentage, as well as an expanded population of $CD21^{low}$ B-cells (12%). Lymphocyte level ranged between 700 and 1100 cells/μl in sequential analyses. Antibiotic prophylaxis with amoxicillin-clavulanate and replacement treatment with recombinant human hyaluronidase-facilitated subcutaneous immunoglobulin (fSCIg) were initiated, therefore limiting further immunological studies. With regard to the lung imaging, although solid data regarding diagnostic and prognostic markers of granulomatous -lymphocytic interstitial lung disease (GLILD) are currently lacking, and considerably variable histopathological findings in GLILD patients have been described, the diagnostic hypothesis of GLILD was considered. Lung biopsy and bronchoscopy, however, were not performed because of the high risk of the procedure due to disease severity and comorbidities, while lung diffusion testing was limited by the patient's poor compliance.

Genetic testing was conducted in accordance with the Helsinki Declaration, after obtaining informed consent from the patient's parents. Sequencing analysis for Kabuki syndrome panel detected a nonsense variant in the *KMT2D* (NM_003482.3) gene: c.8974G>T, p.Glu2992Ter. This variant, confirmed by Sanger analysis, was not reported in the database of human variations GnomAD (https://gnomad.broadinstitute.org/) and was classified as likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria (17).

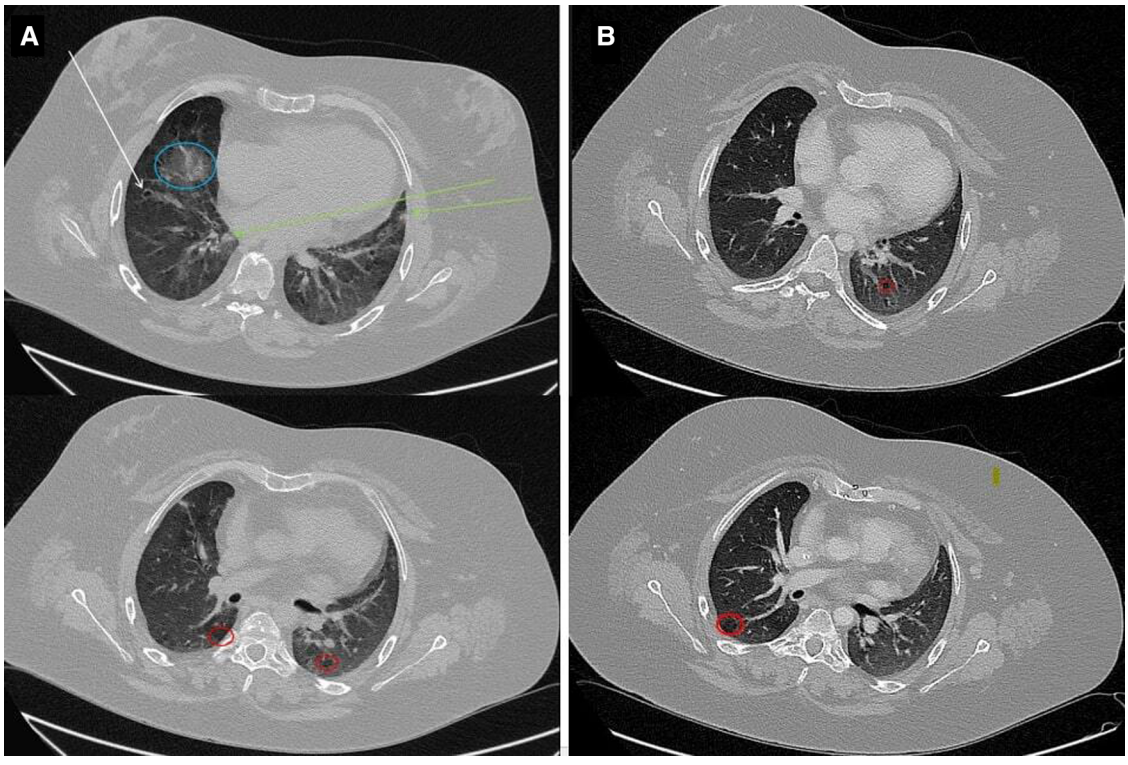

FIGURE 2
Chest HRCT. (A) Images show interstitial lung disease and chronic inflammation. Note the numerous findings, including ground-glass opacity (blue circle), bronchiectasis (white arrow), nodules with blurred edges (green arrows), and regions of air trapping (red circles). (B) Radiological disease improvement with persistence of air-trapping.

At 6-month follow-up evaluation after fSCIg and antibiotic initiation, no infections were reported. Bimonthly fSCIg have been administered at the dosage of 0.4 g/kg. The frequency of infusions was adjusted according to IgG levels and clinical history over time. Pre-infusion IgG levels of 700–800 mg/dl, associated to an optimal clinical outcome, were finally reached with a monthly fSCIg replacement dosage of 0.4 g/kg. Insulin was no longer needed since blood glucose target concentration was reached only by diet. HRCT of the lungs performed during follow-up demonstrated an important decrease of the previously described opacities and nodules, with the sole persistence of air-trapping (**Figure 2B**). Considering the radiological disease improvement obtained with immunoglobulin replacement, the diagnosis of GLILD was eventually ruled out. Hemoglobin level remained at about 12 emsp14;g/dl in the absence of any transfusion and without features of hemolysis. ITP, which initially was partially responsive to high-dose prednisone treatment, improved after immunoglobulin replacement, with a platelet count durably greater than 70,000/μL; relapses, previously documented at any attempt of corticosteroid withdrawal, became fewer and better controlled with low-dose corticosteroids (**Figure 3**). Alternative therapies, including rituximab have been therefore postponed.

Discussion

We hereby report the case of a 23-year-old woman affected by KS presenting with hypogammaglobulinemia, chronic lung disease due to recurrent respiratory infections and immune dysregulation, and several relapses of ES progressively increasing in frequency despite corticosteroid courses.

An international consensus in 2019 defined KS diagnostic criteria (18, 19). The syndrome is characterized by dysmorphic facies (defined by an eversion of the lower lateral eyelids, arched eyebrows with sparse lateral part, depressed nasal tip and prominent ears), postnatal hypotonia, growth deficiency, several skeletal and visceral malformation (including cleft palate, congenital heart defects), dermatoglyphic anomalies and mild to moderate grade of intellectual impairment. Beyond multiorgan anomalies and intellectual disability, autoimmune disorders, including ITP, AIHA, vitiligo and thyroiditis may complicate KS presentation. In KS patients, ITP seems to be the most frequent among immune-mediated cytopenias, but cases of AIHA and/or neutropenia, either combined with ITP or presenting as single-line cytopenia, are also described (7, 12, 20).

Primary autoimmune cytopenias in childhood, especially ITP, are often self-limiting disorders or responsive to first-line therapy (21). Conversely, early-onset, long-lasting, multilineage, refractory cytopenias should strongly suggest an underlying monogenic immune defect (20, 22–24). Consistently with the abovementioned findings, when associated with KS, autoimmune cytopenias, and more specifically ITP, are characterized by severe presentation with chronic or relapsing symptoms, poorly responsive to conventional treatments (13). Moreover, in several patients with KS, non-malignant lymphoproliferation and recurrent respiratory infections, due to hypogammaglobulinemia, have been reported (25, 26).

KS is indeed a paradigmatic example of immunological dysregulation due to mutation in the *KMT2D* or *KDM6A* genes, involved in epigenetic modulation of immune system function. The *KMT2D* gene, also known as *MLL2*, encodes for a histone H3 lysine 4 (H3K4) mono-methyltransferase, essential for cell differentiation and embryonic development (27). Methylation on H3K4 is found in actively transcribed genes. The *KDM6A* gene, also known as *UTX*, encodes for the lysine-specific demethylase 6A linked with demethylation of lysine residues on histone, in particular H3K27, resulting in a gene de-repression. Methylation on H3K27 is associated with transcriptional repression, therefore the action of the *KDM6A* product allows chromatin opening and active gene transcription (28). Mutations of these enzymes cause an impairment of epigenetic activation of certain genes, leading to the distinctive developmental abnormalities of KS. The immune characteristics of KS patients with *KMT2D* mutations may derive from a loss of H3K4 methylation at crucial transcription factors, that finally dysregulates B and T

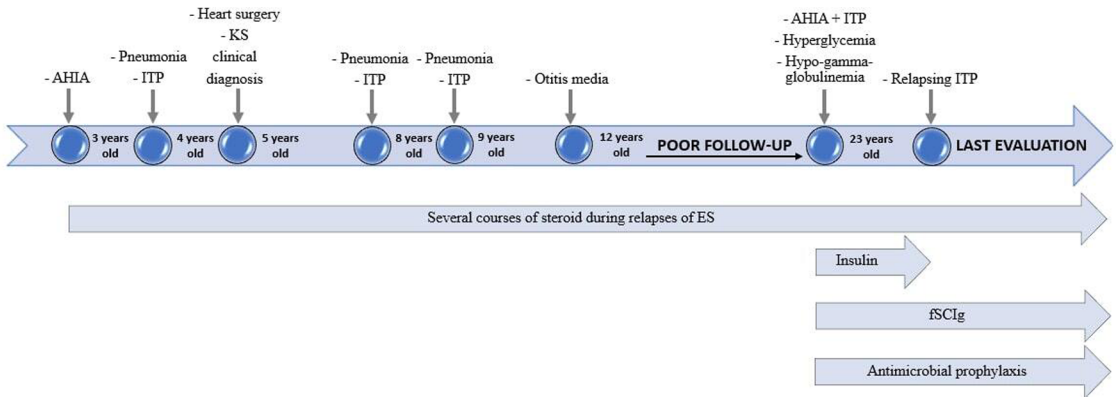

FIGURE 3
Timeline of principal clinical events and therapeutic strategies.

differentiation. A disrupted terminal B-cell differentiation and an impaired somatic immunoglobulin hypermutation have been observed in patients with *KMT2D* mutations, likely as result of impaired epigenetic regulation of the IGH locus. KS patients can in fact present with hypogammaglobulinemia and reduced number of memory B-cells associated to expansion of CD21 B-cell population (29).

Moreover, a clustering of missense mutations in the terminal region of the *KMT2D* gene might increase the risk for autoimmune diseases, depending either on defective regulatory T-cells (Tregs) generation or intrinsic B tolerance breakage (10, 12, 29, 30). Immunodeficiency may worsen with age and more than 80% of KS patients, mostly with *KMT2D* mutations, display hypogammaglobulinemia and diminished memory B-cell populations (7). However, autoimmune diseases or hypogammaglobulinemia may not occur until later in childhood, being often underestimated or undiagnosed for a long time, leading to a substantial diagnostic delay. Our patient's case is paradigmatic, since she was referred to our center several years after the onset of refractory autoimmune disease, while also presenting with severe lung disease, suggesting a long-term, undiagnosed, hypogammaglobulinemia. Immunological study was indeed performed for the first time in this patient in occasion of our first observation.

Susceptibility to infections, especially of middle ear and upper respiratory tract, is common among patients with KS (31). Hearing loss, mainly due to recurrent middle ear infections, occurs approximately in up to 80% of patients, and can be conductive, sensorineural or mixed (1, 32, 33). An early immunoglobulin replacement therapy could therefore have a positive effect on hearing function by preventing recurrent otitis media. It is thus advisable to perform a routine and periodic immunologic evaluation in KS patients, in order to prevent chronic diseases and to reduce morbidity and mortality rate. Moreover, because most of KS cases are reported in the pediatric age, the actual frequency of immune alterations in adults with KS may be underestimated.

In addition, as aforementioned, autoimmune cytopenias in KS are severe and often refractory to conventional treatment with corticosteroids. Thus alternative therapies, including rituximab, sirolimus and mycophenolate mofetil (13, 15, 34) should be considered. Moreover, although glucocorticoid-induced diabetes mellitus generally resolves when corticosteroids are discontinued, periodic blood glucose assessment should always be performed in case of long-term steroid treatment (35) to prevent complications, such as diabetic ketoacidosis, due to the impairment of pancreatic endocrine function. In this scenario, multidisciplinary evaluation coordinated by a case manager and immunological evaluations during follow-up are crucial. In our case, patient's overall clinical condition improved after fSCIg replacement initiation. No infections, as well as a lower recurrence of AIHA and ITP episodes were observed. In conclusion, this case highlights the need of improving awareness in suspecting immune dysregulation in KS patients. Moreover, it is advisable that KS patients undergo immunologic evaluations at diagnosis and during follow-up, aiming to provide adequate treatment and avoid preventable and severe morbidity.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://databases.lovd.nl/shared/variants/0000881406#00023885, 0000881406.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was also obtained from the patient's legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

LL contributed to conception and design of the manuscript. AT, MF, and RP wrote the first draft of the manuscript and collected data. FG, MG, and GA provided and analyzed data. FCon, AM, AS, FCos, and LT supervised, reviewed and wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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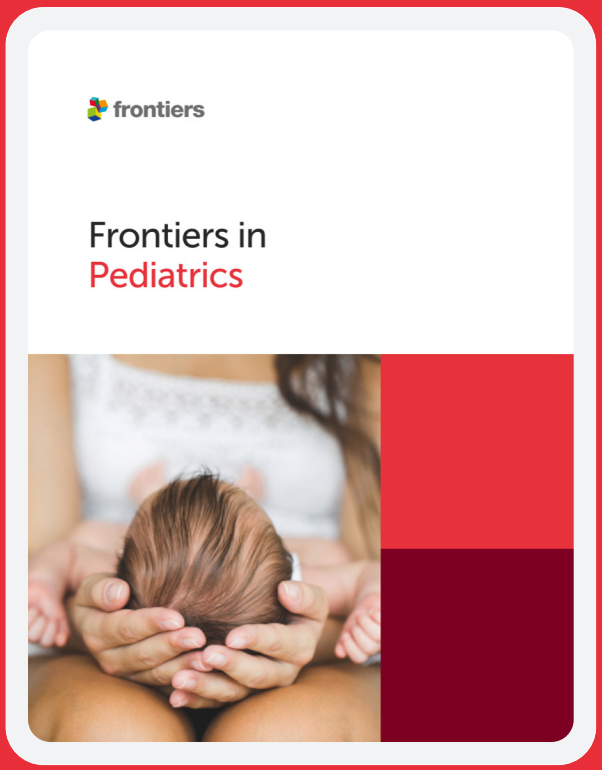

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