Case Report *Iran J Ped Hematol Oncol. 2024, Vol 14, No 3, 244-251*

Pediatric Myelofibrosis: A Rare Entity Posing a Diagnostic Challenge

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Received: 14 June 2023 **Accepted:** 17 November 2023

Abstract

Myeloproliferative neoplasms are clonal hematopoietic stem cell disorders showing proliferation of one or more myeloid lineages. These disorders are characterized by Janus Kinase 2 (JAK2 V617F), Myeloproliferative leukemia (MPL), and Calreticulin (CALR) gene mutations and are seen more commonly in the elderly. These pathognomonic mutations are often absent in children and hence pose a diagnostic challenge. The entire onus of correct diagnosis relies heavily on detailed clinical and laboratory investigations. In the present work, we discuss 4 cases of pediatric myelofibrosis, three of which were secondary to hematolymphoid malignancies, while the remaining one had primary myelofibrosis. Pediatric primary myelofibrosis is a rare entity in children and quite different from adult primary myelofibrosis. The cases in our study show transformation into acute myeloid leukemia, which is associated with an adverse prognosis. Given the rarity of myelofibrosis in children, early and correct diagnosis helps in timely initiation of treatment, which may favorably alter the prognosis.

Keywords: Leukemia, Myelofibrosis, Primary, Secondary

Introduction

Pediatric Myelofibrosis (MF) is a rare disorder. Its prevalence is considered extremely low and has not been well elucidated in the literature. As per global statistics, the incidence of myeloproliferative neoplasms in the younger age group was around 0.82/100,000 patients/year, and for primary myelofibrosis it was 0.53/100,000 patients/year (range, 0.003 to 1.5) (1). In recent times, a case series of 19 patients of pediatric primary myelofibrosis (PMF) has been reported from a single institution(2). There are myriad etiopathological factors attributing to secondary myelofibrosis in children, which include hematological malignancies (acute and chronic leukemias, Hodgkin's and non-Hodgkin's lymphomas, solid tumors), cytopenias, autoimmune disorders, immune deficiency disorders, viral infections, eosinophilia, β-thalassemia and bone marrow failure disorders (3). The entity PMF or idiopathic myelofibrosis includes disorders where no underlying cause for fibrosis is known. These are distinguished by ineffective hematopoiesis, cytopenias, leukoerythroblastosis, proliferation of dysfunctional megakaryocytes, reticulin and/or collagen fibrosis in the bone marrow (BM) and extramedullary hematopoiesis (4). PMF differs from its adult counterpart with respect to clinical presentation, hematological profile, and molecular genetics. The proportion of PMF patients with cytogenetic abnormalities is relatively less (27%) than adults (66%). Among the hematological, erythroid hypoplasia in the bone marrow was a common finding in

[DOI: 10.18502/ijpho.v14i3.15993]

children as compared to adults. *JAK2*V617F is a clonal marker which is a major criteria in the diagnosis of myelofibrosis in adults. However, this has been rarely reported in children with primary myelofibrosis (5). It is important for both clinicians and hematopathologists to be aware of such cases, as pediatric MF may have variable outcomes ranging from spontaneous resolution to a fulminant, potentially fatal course. In the latter, timely intervention with AllogeneicStem Cell Transplantation (ASCT) is therapeutic and may alter the prognosis significantly. This case series highlights four cases of pediatric myelofibrosis. One of these falls within the ambit of primary myelofibrosis, a case of de novo primary myelofibrosis with megakaryoblastic blast transformation having the JAK2 V617F mutation while the other three developed myelofibrosis secondary to hematolymphoid malignancies.

Case studies

Case 1

A two-year-old male, who was already diagnosed case of Down syndrome, presented with gross developmental delay and congestive heart failure. On general physical examination, the child had pallor, hepatomegaly and splenomegaly of 6 cm and 8 cm below costal margin respectively. The child had pancytopenia with severe anemia, hemoglobin (Hb) 2.1 g/dL requiring multiple transfusions, thrombocytopenia (44×10^9/L) and leucopenia (2.61×10^9/L) with the presence of few left-shifted myeloid cells. Few atypical cells which were 2 to 4 times the size of small mature lymphocytes having scant agranular cytoplasm, round nuclei, irregular nuclear membrane, fine chromatin and 1 to 2 nucleoli were seen. BM aspirate smears were hemodiluted.

However, flow cytometric analysis of bone marrow sample revealed 21.5% cells gated in the blast window, 53.1% of which showed CD41 expression. The biopsy showed megakaryocytic hyperplasia with streaming of nuclei (Figure 1A). Megakaryocytes displayed hypolobation with dispersed chromatin and atypia. Few atypical cells with a high N/C ratio, vesicular chromatin and prominent nucleoli were seen. These atypical cells were positive for PAS stain and CD61 (Figure 1). The reticulin stain showed Grade 3 fibrosis (Figure 1C). On the basis of clinical features, flow cytometric analysis and biopsy findings a diagnosis of myeloid leukemia associated with Down's syndrome and myelofibrosis was made.

Case 2

A 2-year-old male child presented to some other centre with a complaint of fever (on-off) for 2 months with no other significant history. General physical examination revealed pallor and bony tenderness in the limbs. There was mild hepatosplenomegaly but no lymphadenopathy. CT scan revealed multiple ill defined permeative bony destructive lesions. He had bicytopenia with low Hb and platelet count. Anaemia was refractory to repeated blood transfusions. The child was referred to our centre for further evaluation. Complete blood count (CBC) revealed normocytic normochromic anaemia with Hb 7.1 g/dL, Mean corpuscular volume (MCV) 95.6 fL, Mean corpuscular hemoglobin (MCH) 32.9 g/dL, normal Total leucocyte count (TLC) 7.54×10^9/L and thrombocytopenia 8×10^9/L. Peripheral smear showed leucoerythroblastic blood picture with the presence of few nucleated red blood cell (RBCs), a left shift in myeloid series and few atypical cells (11%) which were 3-4 times the size of small mature lymphocytes, with a high N/C ratio, having scant agranular basophilic cytoplasm with blebbing at places. The nuclei were round to oval with fine chromatin and 1-2 nucleoli. Repeated bone marrow aspirates were hemodiluted, however showed few atypical cells. Flow cytometric analysis of

[DOI: 10.18502/ijpho.v14i3.15993]

the BM aspirate revealed 30.3 % of cells gated in blast window in a CD45/SSC plot. The cells were positive for CD 41 and negative for MPO, HLA-DR, CD 34, CD 13, CD 33, CD 79a, CD 10, suggestive of megakaryoblasts. The BM biopsy showed streaming of hematopoietic precursors along with focal clustering of atypical megakaryocytes/ megakaryoblasts of variable sizes with clumped chromatin and 1-2 nucleoli. PAS stain was positive in dysplastic megakaryocytes/megakaryoblasts. The reticulin stain showed grade 3 fibrosis. On immunohistochemistry (IHC), these cells were positive for CD 61, hence confirming the presence of megakaryoblasts. Thus, the final diagnosis of paediatric myelofibrosis with megakaryoblastic crisis was made. On cytogenetic analysis JAK-2 mutation was present. The patient was referred to a higher oncology center for further management where he succumbed to the disease.

Case 3

A 7-year-oldfemale child presented with pain in the abdomen , abdominal distension and fever for 3 months. Per abdominal examination revealed massive splenomegaly reaching up to the right iliac fossa and hepatomegaly of 4 cm below costal margin. The hemogram revealed marked leucocytosis (132.24×10^9/L) with presence of 10% blasts having lymphoblast-like morphology, a myeloid bulge, basophilia (5%) and thrombocytopenia (30×10^9/L). Thus, provisional diagnosis of blastic transformation of Chronic Myeloid Leukaemia (CML) was considered. Reverse transcriptase (RT-PCR) revealed Major breakpoint cluster region (M-BCR) fusion protein BCR-ABL ex14-ABL ex2(b3a2):472bp which confirmed the diagnosis. BM aspiration attempted, resulted in dry tap. A BM trephine biopsy was taken. Imprint smears showed clusters of blasts with abovementioned morphology (Figure 1D). The biopsy showed cellular marrow spaces with clusters of blasts and streaming of nuclei. (Figure 1E). On the reticulin stain Grade 3 fibrosis was demonstrated. On IHC, clusters of blast cells were positive for CD34, CD10, CD 19, CD20, CD79a and Pax5 (Figure 1F). These were negative for T-cell and myeloid markers. Flow cytometry performed on the peripheral blood showed blasts with expression of CD34, Tdt, HLA-DR, cCD79a and coexpression of CD10 and CD19. These were negative for CD3, CD5, CD7, cCD3, MPO, CD13, CD33, CD117, CD14. Hence, a final diagnosis of lymphoid blast transformation in Chronic Myeloid Leukemia with associated myelofibrosis was made. She was given Imatinib 300 mg/m^2 once daily and treated according to Indian Childhood Collaborative Leukemia Protocol (ICiCLe). (6) Child is responding well, Day 35 Minimal Residual Disease (MRD) was negative.

Case 4

A 13 year old male child presented with fever, weight loss, loss of appetite for 3 months and prior history of Koch's contact. Per abdominal examination revealed hepatosplenomegaly of 4 cm and 14 cm below costal margin respectively. Bilateral cervical lymph nodes were enlarged and chest X-ray showed mediastinal lymphadenopathy. Lactate dehydrogenase (LDH) levels were raised (1024 U/L). Complete blood counts revealed pancytopenia (Hb 7.9 g/dL, TLC 2.14×10^9 /L, Platelet count 42×10^9/L) whereas BM aspirate done at some other centre showed few Reed Sternberg like cells. Provisional diagnosis of Hodgkin's lymphoma, Non-Hodgkin's lymphoma and acute leukemia were considered and the case was referred to us for further evaluation. BM aspirate smears from both the right and left sides were grossly hemodiluted. Imprint smears were cellular; however showed

[DOI: 10.18502/ijpho.v14i3.15993]

many degenerated bare nuclei. BM biopsy sections showed cellular spaces with fibrosis and the presence of large atypical cells which were 2-4 times the size of small mature lymphocyte with scant to moderate amount of cytoplasm. The nuclei were large with vesicular chromatin and a single prominent eosinophilic nucleolus. These cells were negative for CD15. There was streaming of nuclei with presence of coarse fibre network which on Reticulin stain demonstrated Grade 3 fibrosis. A final diagnosis of marrow infiltration by Non Hodgkin's lymphoma (NHL) with secondary myelofibrosis was suggested. Later the lymph node biopsy confirmed the diagnosis of T-cell/histiocyte-rich large B-cell Lymphoma (TCRBCL) type of NHL.

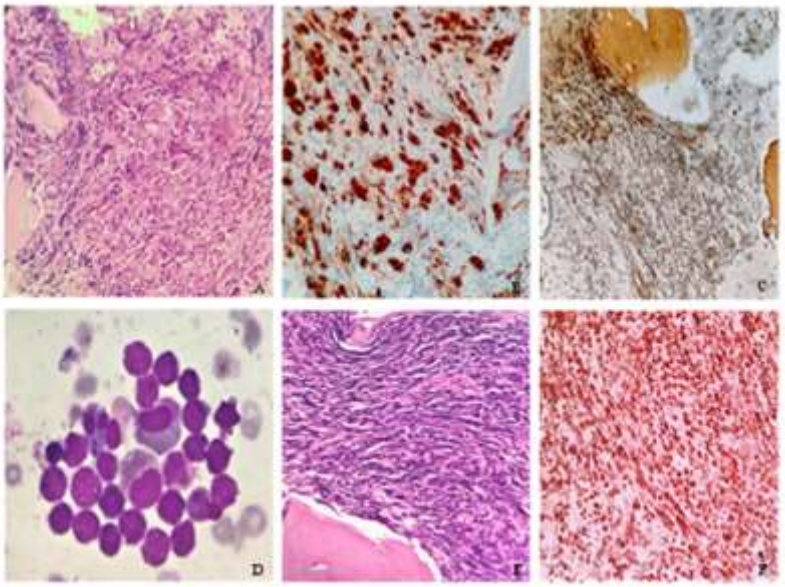

Figure 1A: Trephine biopsy showing megakaryocytic hyperplasia with hypolobated and atypical megakaryocytes and few interspersed atypical cells. [H&E, 400X]
Figure 1B: Atypical megakaryocytes/megakaryoblasts showing CD61 positive expression. [CD61, 400X]
Figure 1C: Grade 3 fibrosis demonstrated on Reticulin stain [Reticulin stain, 400X]
Figure 1D: Bone marrow imprint smears showing cluster of atypical cells with scant agranular basophilic cytoplasm, round to oval nucleus , high N/C ratio and 1-2 nucleoli. [Giemsa, 1000X]
Figure 1E: Trephine Biopsy showing nuclear streaming and fibrosis [H&E, 400X]
Figure 1F: Blasts positive for PAX5 in lymphoid blast transformation of Chronic Myeloid Leukaemia [PAX5, 400X]

[DOI: 10.18502/ijpho.v14i3.15993]

Discussion

Paediatric Primary Myelofibrosis is an uncommon entity and is usually seen in children under 3 years of age with female preponderance. (7)

Primary Myelofibrosis can also be further classified as chronic PMF and acute PMF. Acute PMF is relatively more common in children and is usually associated with Acute Megakaryoblastic Leukemia (AMKL) and Acute Panmyelosis with Myelofibrosis (APMF), which have been recently described as part of the spectrum of Acute Myeloid Leukemia with Myelofibrosis. (8)

There is a variant of AMKL in Down's syndrome called Acute Myelofibrosis in children with Down's syndrome which shows the presence of bizarre megakaryocytes and blasts which clinically resemble acute leukemia. The current World Health Organisation (WHO) describes blasts with round to slightly irregular nuclei and a moderate amount of basophilic cytoplasm with or without cytoplasmic blebs. Bone marrow histology shows generalized fibrosis, a markedly increased reticulin, with or without increased megakaryocytes, dysplastic small forms in clusters and micromegakaryocytes (9). Case 1 showed fibrosis as well as blast cells in the peripheral blood and bone marrow.

It is difficult to diagnose BCR-ABL1 negative MPNs in children since the mutations that are pathognomonic for these disorders like JAK2 V617F and MPL mutations, are rarely found or even absent, as reported in some children with PMF. Thus, it is primarily a diagnosis of exclusion thatis arrived at after excluding other more commoner differentials such as AML-M7 (acute myelofibrosis), acute megakaryoblastic leukemia (AMKL), myelodysplastic syndrome (MDS) with myelofibrosis or myelofibrosis with acute lymphoblastic leukemia (ALL) (6).

The myeloproliferative neoplasm (MPN) subtype determines the leukemic evolution of the disease, which is highest in PMF, i.e. at approximately 10–20% over 10 years (10).

Several risk factors have contributed to the leukemic evolution in BCR-ABL1-negative MPNs which include advanced age, leukocytosis, exposure to myelosuppressive therapy, cytogenetic abnormalities and an increased number of mutations in genes associated with myeloid neoplasms (11).

Few independent risk factors responsible for leukemic transformation in PMF include peripheral blood blast count >3% and platelet count $<100 \times 10^9$ /L (12). The myeloid lineage is commonly involved in the Blast-phase MPNs, and CD34 immunostain can be employed in highlighting the individual blast cells, their clusters, and their localisation.

MPN undergoing blast transformation may be confused with primary de novo AML (AML-DN); a clear distinction between the two can be made on the basis of morphological and cytogenetic characteristics.

Case 2 showed 11% blasts in peripheral blood and platelet count was markedly reduced to $8x10^9$/L. An increased risk of leukemic transformation in PMF could be assessed by leukocytosis and increased frequency of RBC transfusion. Our patient was refractory to repeated blood transfusions ; however, leucocyte count was within normal limit (7.4×10^9/L). The most common bone lesion associated with MF is osteosclerosis. However, osteolytic lesions were seen in the present case which are uncommon in hematological malignancies (13). The most important risk factors for leukemic evolution in PMF include unfavourable karyotype together with thrombocytopenia, where a higher risk has been associated with the triple-negative molecular status as well as mutations

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involving epigenetic modifiers and spliceosome machinery (14). The patient was referred to a higher centre for extensive cytogenetic and molecular work up, however, he succumbed to the disease before investigations could be undertaken.

The proportion of patients with CML-AP and CML-BC within pediatric CML is documented as 1.9% and 4.4 % respectively in CML-Paed-II Trial (15). Pediatric CML is different from adult CML. The median WBC count in pediatric CML was significantly higher than in adult CML and splenomegaly as seen in 76% patients had median size of 8 cm below the costal margin (16). Case No. 3 in present series had massively enlarged spleen reaching up to the iliac fossa, with marked leucocytosis having myeloid bulge, basophilia, and presence of 10% lymphoblasts. There was thrombocytopenia (30×10^9/L), which is a rare finding that has been reported only in 5.5% patients of CML (17).

CML Lymphoid Blast Crisis in children poses a diagnostic dilemma as it is hard to differentiate from Ph+ ALL and AML with BCR-ABL1. Marked splenomegaly, basophilia, myeloid bulge, and p210 fusion gene help in making the distinction.

Myelofibrosis associated with CML is typically described in adults and has been reported as 26% in a large study comprising 614 patients with adult CML (18). Exhaustive literature review has rarely revealed a case of CML with lymphoid blast crisis and myelofibrosis in a child, barring a case of CML with megakaryoblastic blast crisis with fibrosis (19).

Bone marrow involvement in Diffuse Large B-cell Lymphoma (DLBCL) is seen in 20-30% cases while in TCRBCL, a distinctive form of DLBCL, it is 25-60% (20). The case 4 was diagnosed as TCRBCL which is rarely reported in pediatric population (21) This entity poses a diagnostic challenge as it morphologically resembles nodular lymphocyte predominant Hodgkin's lymphoma and peripheral T-cell lymphoma. However, TCRBCL is defined by non-neoplastic T-cells and variable numbers of histiocytes. The neoplastic large cells of TCRBCL can be distinguished from classical Hodgkin's lymphoma by their CD20+, CD30- and CD15- phenotype. In a large study comprising 375 lymphoma patients with median age 62 years, 25 (6.6%) had marrow fibrosis frequently associated with low grade non-Hodgkin's lymphoma. None of the cases had JAK2V617F mutation (22). The present case also lacked this mutation.

Conclusion

Pediatric myelofibrosis is a unique clinical entity with characteristic clinical and pathological features, which is primarily a diagnosis of exclusion. No definitive treatment apart from haematopoietic stem cell transplantation (HSCT) has proven to be therapeutic and if correctly diagnosed may have favourable outcome after HSCT. Thus, early and correct diagnosis is the mainstay of treatment. Rare cases such as these add considerably to the research pool and may make inroads for further genetic study employing next-generation sequencing.

Ethical Consideration

No ethical rule violation was done. Written informed consent was taken from parents of the children for publication. No image or identifying features have been published.

Author's contributions

All authors contributed to the study conception and design.

Acknowledgement

We would like to extend our gratitude to the Department of Pathology and Department of Pediatrics, Lady Hardinge Medical College & Associated Hospitals for their constant support and guidance.

[DOI: 10.18502/ijpho.v14i3.15993]

Funding
No funding was received to assist with the preparation of this manuscript

Conflict of interest
The authors have no conflicts of interest to declare that are relevant to the content of this article.

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[DOI: 10.18502/ijpho.v14i3.15993]

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