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Case Report

Identification of a Novel Unbalanced t(4;8) (q12;q24) in Paediatric Acute Myeloid Leukemia M2 with AML-ETO Negative Patient by Fluorescence in Situ Hybridization Technique

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Abstract

Identification of chromosomal abnormalities in patients with Acute Myeloid Leukemia (AML) has contributed substantially to our current understanding of the molecular pathogenesis underlying leukemogenesis, and risk-stratification. Based on molecular abnormalities both influences treatment strategies and aids in determining prognosis. While over 300 established mutations have been documented in AML, the enhanced availability of genetic analysis and the increase in awareness of uncommon chromosomal translocations have made it possible for rare, apparently unique translocations to become recognized and to ultimately gain prognostic significance. Hence, we present a case of AML with a novel, t(4;8) involving breakpoints previously undescribed. Although the patient required second induction, first remission was ultimately achieved. While the prognostic significance of this translocation is not fully elucidated, it is our hope that documentation of this patient's presentation will help to characterize the significance of a yet undefined cytogenetic abnormality in AML

Keywords: myeloid leukemia; situ hybridization technique; leukemogenesis

Introduction:

The most common form of the acute leukemias is an Acute Myeloid Leukemia (AML), with nature of complexity, heterogeneity and with marked genetic, epigenetic, and phenotypic variation [George M. Jehaa, Tiffany Wesley, Vince D. Cataldo 2020]. In most cases of AML, Chromosomes of malignant cells harbour specific, non-random, and often recurrent abnormalities which have important implications in terms of clinical and pathological presentation, prognosis, and therapeutic response. AML in children is a rare and heterogeneous disease, with an incidence of 7 cases per million children younger than 15 years. Survival rates increased upt to 70% due to intensive therapy in combination with effective supportive therapy in high-income countries, [Amanda de Lourdes Nunes et al, Ursula Creutzig et al 2012].

According to cytogenetic abnormalities chromosomes are grouped in three prognostic categories: favorable, intermediate, and adverse. Some of these abnormalities are common and others are rare. Rare cytogenetic abnormalities include aberrations of chromosomes 3, del (5q),- 5 and − 7 .Cytogenetic and molecular data are recognized as the most valuable prognostic factors in AML both in National Comprehensive Cancer Network (NCCN) and European Leukemia Net (ELN) risk stratification models [ANUDISHI TYAGI et al 2018 & Matahi Moarii et al 2017].

In this case, we describe a report of AML-M2 patient with cytogenetic and FISH study using Bone marrow sample. Cytogenetic results revealed that there was a translocation between chromosome 4 and 8 in all 20 metaphases analyzed. Patient's karyotype analysis revealed 46, XY, t(4;8)(q12;q24)[20]. Karyotype results and FISH results were compared using Whole Chromosome Paint (WCP) FISH probes and Locus Specific Identifier (LSI) *RUNX1/RUNX1T1*fusion Dual color dual fusion (DCDF) probe.

Case Details:

We report a patient with AML-M2 who developed t(4;8)(q12;q24) prior to the course of therapy. A 2-year-old male child suffering from low grade fever, weakness, join swelling of bilateral dorsum of hand to Gujarat Cancer and Research Institute, Ahmadabad, India.

On admission the laboratory investigations revealed White Blood Cell count 67.63 $X10^3$/cmm, Red Blood Cell count 4.34$X10^6$/cmm, Haemoglobin-8.8 gm/dl, and Platelets 231 $X10^3$/cmm, Myelocyte 14 %, Polymorphs 56%, and Lymphocytes 8%, Eosinophils 3%. Bone marrow

report showed hypercellular with altered M: E ratio. PAS Stain Negative, Sudan Black Positive, Blast Cells 45 %, Promyelocytes 5 %, Myelocytes-M 10 %, Metamyelocytes- 2 %, Band Cells 2 %, Polymorphs- 4 %, Eosinophils- 3 %, Lymphocytes- 4 %, Inter Normoblasts5 %, Late Normoblasts 8 %, Reticulum Cells- 2 %. It shows proliferation of blast cells (45%). These blasts are medium to large in size, having high N:C ratio, fine chromatin & moderate amount of cytoplasm, having fine granules in few. Myeloid & erythroid precursors are suppressed. Megakaryocytes are present. RBCs are Hypochromic microcytic. Marked leukocytosis with blast cells. Blast-11, Promyelocyte-05, Myelocyte-14, P-56, L-08, M-03, E-03. Platelets - Adequate. Diagnosis: Acute Myeloid Leukemia (AML-M2/M4). X-Ray Reports of chest was normal. Ultra-sonography reports of Abdomen pelvis were normal. FLT3/ITD mutation by RFLP method was negative from peripheral blood sample.

The patient was treated with Cytarabine 100 mg, Etoposide 100 mg, Filgrastim 300 mg, Mercaptopurine 50mg, Vincristine 1 mg, Doxorubicin 10mg and achieved remission.

Present study was approved by the institutional review board and patients' general consent was taken.

Materials and Methods:

Conventional Cytogenetics

Bone marrow sample was collected ascetically in Sodium Heparinized vaccuate. For conventional cytogenetic study, short term culture was carried out as per standard protocol and slides were banded using Giemsa Trypsin G banding technique. Good morphology metaphases were captured in Zeiss automatic karyotyping system and analysis using IKAROS software and karyotype description was done using ISCN 2016 guidelines.

Fluorescence in situ hybridization (FISH):

Using Epi-fluorescence microscope (AXIO Imager.Z2, Zeiss, USA) equipped with appropriate filter sets, image capturing, and processing were carried out using an ISIS FISH imaging system (MetaSystems, Germany). FISH was performed using *RUNX1/RUNX1T1* LSI probe. In LSI *RUNX1/RUNX1T1* probe, *RUNX1* gene was tagged with Spectrum green and *RUNX1T1* gene tagged with Spectrum Orange.

Results:

Conventional Cytogenetic:

Conventional chromosome analyses at diagnosis of GTG banded metaphase were carried out. Total 20 metaphases were karyotyped. All metaphases showed 46,XY,t(4;8)(q12;q24)[20] (Figure.1 A).

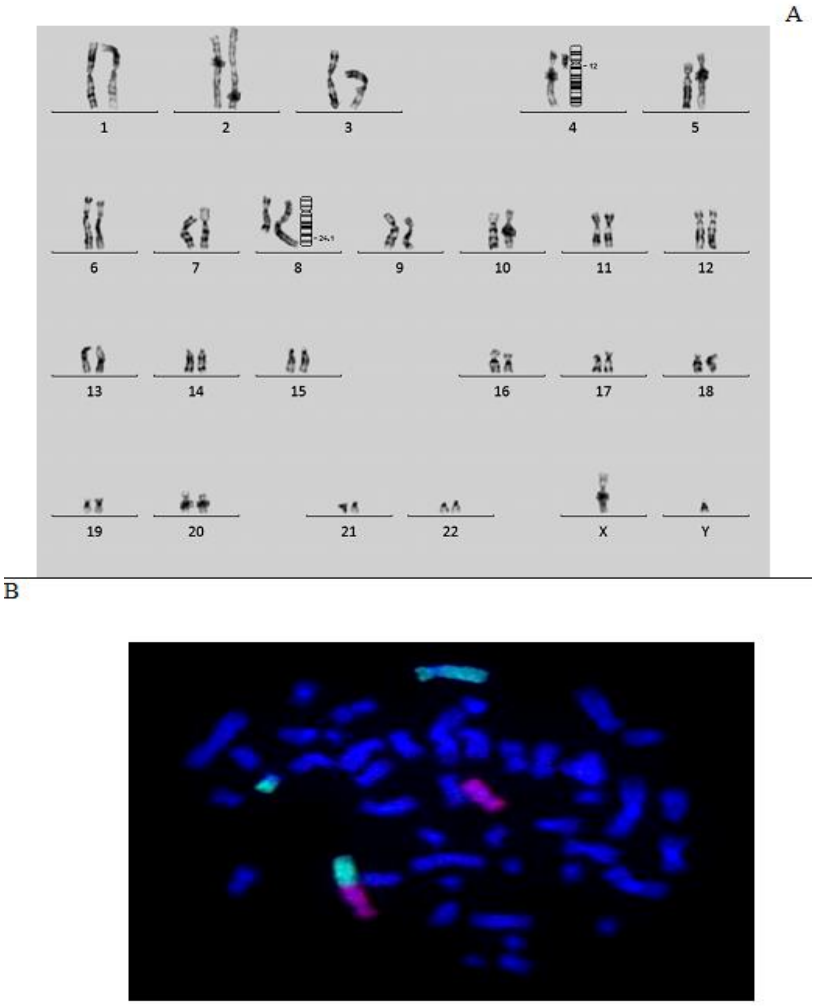

Figure1: *(A) Conventional cytogenetic results of GTG banded karyotype showing t(4;8)(q12;q24) (B) Fluorescence in situ Hybridization results using Whole chromosome paint FISH probes. Spectrum orange #8 and spectrum green #4. Three green chromosomal materials indicate unbalanced translocation between chromosome 4 and 8. Part of chromosomal material from chromosome 4 is observed on q arm of chromosome 8.*

FISH:

FISH results with *RUNX1/RUNX1T1* probes results revealed metaphases with 2O2G signals which was observed which showed that there was no fusion for *RUNX1/RUNX1T1* gene (Figure.2). FISH result was nuc ish(*RUNX1/RUNX1T1*) X2[200].

As this is an unknown translocation in AML, a gene specific probe for this specific translocation was not utilized. So, the WCP FISH was carried out to determine the nature of the translocation. The WCP FISH probes for chromosome 4 Spectrum Green (SG) and chromosomes 8 with Spectrum Orange (SO) were applied on metaphase cells as per manufactures instructions. Results of WCP FISH revealed that there was unbalanced translocation between chromosome 4 and 8. Part of q arm of chromosome 4 was observed attached to the q arm of chromosome 8 and confirmed that t(4;8)(Figure 1B).

Discussion:

Conventional cytogenetics can detect structural and numerical cytogenetic abnormalities in 70%-80% of children with AML. Certain fusion genes, products from cryptic translocations, or loss of chromosome material can only be reliably detected using FISH. The diagnostic cytogenetic workup in children and adults is similar. The most frequent chromosomal abnormalities in children with AML include t(8;21)(q22;q22), inv(16)(p13.1q22) (together referred as core binding factor [CBF]-AML, t(15;17)(q22;q21)/*PML-RARA*, and 11q23/*MLL*-rearranged abnormalities (up to 25%), which together account for approx. 50% of pediatric AML, a much higher frequency than in adults. Additional abnormalities that are more predominant in pediatric AML are, for example, t(1;22)(p13;q13)/*RBM15(OTT)-MKL1(MAL).* Further types are the cryptic abnormalities t(7;12)(q36;p13)/*ETV6(TEL)-HLXB9(MNX1*), which are strongly associated with +19 and t(5;11)(q35;p15.5)/*NUP98-NDS1*, predominantly found in cytogenetically normal AML (CN-AML). However, both translocations have not yet been included in the WHO 2008 classification [Creutzig et al 2012].

In present study we come across novel translocation (4;8)(q12;q22). The gene present on 4q12 is PDGFRA & several chromosomal rearrangements generating fusion genes causing PDGFRA activation have been described in a variety of uncommon hematologic disorders that are often accompanied with a related condition called hypereosinophilic syndrome. These rearrangements activate PDGFRA by fusion to various partner genes: STRN (2p24) in the , FIP1L1 (interstitial 4q12 deletion), CDK5RAP2 (9q33) in the , KIF5B (10p11) in the , ETV6 (12p13) in the , and BCR (22q11). [Baxter et al., 2002; Gotlib et al., 2008]. PDGFA has transmembrane receptor protein tyrosine kinase activity and acts as a cell-surface receptor for members of the platelet-derived growth factor family: PDGFA, PDGFB and PDGFC, which are mitogens for fibroblasts and cells of mesenchymal origin. It plays an essential role in the regulation of many biological processes including cell proliferation, survival, differentiation, and cell migration & plays a role in platelet activation, wound healing, and angiogenesis [Demoulin et al., 2012; Heldin et al., 20103]. MYC gene located on 8q24 and present predominantly in the nucleus & expressed in almost all proliferating cells in embryonic and adult tissues; expression correlates with cell proliferation; abnormally high expression is found in a wide variety of human cancers. Its main role in Burkitt lymphoma, diffuse large cell Lymphoma, multiple myeloma, lung cancer, breast cancer colon cancer and prostate cancer & it's expressed by different mechanisms like chromosomal translocation, amplifications, point mutations, epigenetic reprogramming, enhanced translation and increase protein stability. In 70% of cancers its expression is deregulated or elevated [Adrian Krygier et al 2020].

Outcome is of paediatric patients mainly depends on the initial response to treatment and chromosomal aberrations. Combinatorial therapy of Anthracycline- and Cytarabine-containing chemotherapy and stem cell transplantation in selected genetic high-risk cases or slow responders. Relapse observed in, ~30% of all pediatric AML patients, whereas death is observed in 5%–10% of the patients due to disease complications or the side-effects of the treatment [Jasmijn D. E. de Rooij et al 2015]. Our patient received standard induction therapy and Etoposide and 6 thioguanine and achieved complete remission. Standard induction therapy comprises 3 days of an Anthracycline (e.g. Daunorubicin at least 60 mg/m2, idarubicin 10-12 mg/m2, or the Anthracenedione Mitoxantrone 10-12 mg/m2) and 7-10 days of Cytarabine (100-200 mg/m2 continuously or twice daily intravenously, ie, "3_7" or "3_10"). With these regimens, ~85% of children and adolescents achieve complete remission (CR). Although a third drug, such as Etoposide or 6-thioguanine, is commonly included in induction, their benefit has not been proven.

Age is an important factor among children, as indicated by the age-specific FAB/WHO subtypes. Infant AML was historically defined as AML occurring in children under 1 year of age, but it has now been extended to include all children under 2 years, because they share the same clinical and biological profiles [Julie Quesada et al 2021]. For example, they include a higher proportion of acute megakaryoblastic leukemia (AMKL, M7), whereas certain cytogenetic abnormalities are exclusively identified in this age group, such as t(1;22)(p13;q13)/RBM15- MKL[Creutzig, U. et al 2012; De Rooij et al 2015; Harrison et al 2010; Von Neuhoff et al 2010].

Till date in Mitelman database, **71,734**cases and **32,721**gene fusion for the chromosomal aberration have been reported [**July 15, 2021**]. Moreover, t(4;8)(q12;q24) with different regions have been observed in 11 cases in different malignancies but not observed in AML-M2. So, our case is the novel finding. Although this patient required second induction, first remission was ultimately achieved. While the prognostic significance of this translocation is not fully elucidated, it is our hope that documentation of this patient's presentation will help to characterize the significance of a yet undefined cytogenetic abnormality in AML.

To identify the exact breakpoints, present in AML and MDS and to identify fusion genes because of the unbalanced translocation array-CGH analysis required.

Conclusion:

Targeted therapy may enhance anti-leukemic efficacy and minimize treatment-related morbidity and mortality but requires detailed knowledge of the genetic abnormalities and aberrant pathways involved in leukemogenesis. So, the presence of certain genetic abnormalities, usually detected by Fluorescence in Situ Hybridization (FISH) analysis, Polymerase Chain Reaction (PCR) and next generation sequencing makes it possible to risk stratify patients at the time & these efforts towards future personalized therapy in pediatric AML.

References:

1. M. Jehaa, Tiffany Wesleya, Vince D. Cataldob. (2020). Novel Translocation in Acute Myeloid Leukemia: Case Report and Review of Risk-Stratification and Induction Chemotherapy in Patients with Acute Myeloid Leukemia George d Journal Hematology. 9(1-2): 13-17.
2. Anudishi Tyagi, Raja Pramanik, Shilpi Chaudhary, Anita Chopra & Sameer Bakhshi. (2018). Cytogenetic Profiles of 472 Indian Children with Acute Myeloid Leukemia Indian Pediatrics. (55): 469-473.
3. Riccardo Masetti, Francesca Vendemini, Daniele Zama, Carlotta Biagi, Andrea Pession and Franco Locatelli. (2015). Acute

myeloid leukemia in infants: biology and treatment. Frontiers in Pediatrics.
4. Jasmijn D. E. de Rooij, C. Michel Zwaan and Marry van den Heuvel-Eibrink. (2015).Pediatric AML: From Biology to Clinical Management. Journal Clinical Medicine. 127-149.
5. Julie Quessada, Wendy Cuccuini, Paul Saultier, Marie Loosveld, Christine J. Harrison, and Marina Lafage-Pochitaloff Genes (2021). Cytogenetics of Pediatric Acute Myeloid Leukemia: A Review of the Current Knowledge. (12):924.
6. Grimwade D, Walker H, Oliver F, et al. (1998). On behalf of the Medical Research Council Adult and Children's Leukaemia Working Parties. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered the MRC AML10 trial. 2322-2333.
7. Slovak ML Kopecky KJ Cassileth PA, et al. (2000). Karyotypic analysis predicts outcome of preremission and post remission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. 4075-4083.
8. Schoch C, Haferlach T, Haase D, et al. (2001). Patients with de novo acute myeloid leukaemia and complex karyotype aberrations show a poor prognosis despite intensive treatment: a study of 90 patients. British Journal of Haematology.112 (1):118-126.
9. Byrd JC, Mrózek K, Dodge RK, et al. (2002). Pre-treatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). 4325-4336.
10. Suciu S, Mandelli F, de Witte T, et al. (2003). Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMA AML-10 trial. 232-1240.
11. Haferlach T, Kern W, Schoch C, et al. (2004). A new prognostic score for patients with acute myeloid leukemia based on cytogenetics and early blast clearance in trials of the German AML Cooperative Group. Haematologica.89(4):408-418.
12. Cornelissen JJ, van Putten WL, Verdonck LF, et al. (2007). Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom?. 3658-3666.
13. Creutzig U, Heuvel-Eibrink M.M.V.D, Gibson B, Dworzak M N, Adachi S, De Bont E, Harbott J, Hasle H, Johnston D, Kinoshita A, et al. (2012). Diagnosis and management of acute myeloid leukemia in children and adolescents: Recommendations from an international expert panel. 120:3187-3205.
14. De Rooij J DE, Zwaan CM, Van den Heuvel-Eibrink M. (2015). Pediatric AML: From biology to clinical management. Journal of Clinical Medicine. 127-149.
15. Harrison CJ, Hills R, Moorman A, Grimwade DJ, Hann I, Webb DKH, Wheatley K, De Graaf S S N, Berg E V D, Burnett A K, et al. (2010). Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment Trials AML 10 and 12. Journal of Clinical Oncology.28:2674-2681.
16. Von Neuhoff C, Reinhardt D, Sander A, Zimmermann M, Bradtke J, Betts DR, Zemanova Z, Stary J, Bourquin J P, Haas O A, et al. (2010). Prognostic impact of specific chromosomal aberrations in a large group of pediatric patients with acute myeloid leukemia treated uniformly according to trial AML-BFM 98. Journal of Clinical Oncology. (28); 2682-2689.
17. Mitelman F, Johansson B and Mertens F. (2021). Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer.
18. Jordan J, Simons A, Schmid M. (2016). International standard for chromosomal nomenclature 2016 guidelines McGowan- ISCN 2016.Publisher Karger.
19. Matahi Moarii , Elli Papaemmanuil. (2017). Classification and risk assessment in AML: integrating cytogenetics and molecular profiling. 37-45.
20. The t (4; 22) (q12; q11) in atypical chronic myeloid leukemia fuses BCR to PDGFRA.
21. Baxter EJ, Hochhaus A, Bolufer P, Reiter A, Fernadez JM, Senent L, Cervera J, Moscardo F, Sanz MA, Cross C. (2002). Human Molecular Genetics. 11(12):1391-1397.
22. Gotlib J, Cools J. (2008). Five years since the discovery of FIP1L1-PDGFREA: What we have learned about the fusion and other molecularly defined eosinophilias.1999-2010
23. Demoulin JB, Montano-Almendras CP. (2012). Platelet -Derived Growth factos and their receptors in normal and malignant Hematopoiesis. American Journal of Blood Research.2 (1):44-56.
24. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. (2003). PDGFRA activation mutations in gastrointestinal stromal tumors. 299(5607): 708-710.
25. Adrian Krygier, Dagmara Szmajda-Krygier, Aleksandra Sałagacka-Kubiak, Krzysztof Jamroziak, Marta Żebrowska-Nawrocka & Ewa Balcerczak (2020). Association between the CEBPA and c-MYC genes expression levels and acute myeloid leukemia pathogenesis and development. Medical Oncology. 37:109.

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