banding cytogenetics. In this work, we describe the clinical and molecular data of four pediatric patients with BCP-ALL iAMP21, showing the importance of RUNX1-RUNX1T1 and ETV6-RUNX1 FISH probes in screening this high-risk entity. **Material and methods:** This collaborative study included four children with BCP-ALL iAMP21. The peripheral blood and Bone Marrow (BM) samples were referred to the cytogenetics laboratory at the Brazilian National Cancer Institute between 2013 and 2021. We performed G-banding and FISH experiments in interphase nuclei and metaphase spreads. We used commercial Locus-Specific (LSI) ETV6-RUNX1, RUNX1-RUNX1T1, and centromeric 13/21 FISH probes. **Results:** Using the FISH technique with LSI ETV6-RUNX1 and RUNX1-RUNX1T1 probes, we identified the derivative chromosome 21 and the precise number of RUNX1 signals for each patient. It also helped disclose whether the RUNX1 amplifications were intra- and or extrachromosomal. **Discussion:** The intrachromosomal amplification of chromosome 21 is an uncommon high-risk chromosomal aberration in pediatric B-ALL. iAMP21 can be elusive, sometimes escaping detection by banding cytogenetics. Thus, confirmation by molecular techniques is necessary. Besides, recent studies suggest a relapse risk associated with therapy intensity, emphasizing the importance of a rapid and early detection of iAMP21 for appropriate risk stratification and therapeutic intervention. The FISH approach used through our experimental design has proved efficient in the early detection of iAMP21 in all patients. Importantly, it helped determine the diagnosis of iAMP21 48 hours after initial diagnosis. Our approach can also be valuable in monitoring patients for Minimal Residual Disease (MRD). According to the literature, those who continuously present MRD should be eligible for BM transplantation. **Conclusion:** In summary, the flexibility of FISH probes enabled the rapid detection of this high-risk neoplasm. Besides, the precise characterization of iAMP21, including the aberrant chromosomes 21 and extra RUNX1 signals, was important to guide treatment strategies and the monitoring of patients. In addition, we reinforce the importance of investigating the heterogeneity of iAMP21 marker chromosomes, their molecular complexity, and genomic association with disease progression. Novel genetic findings may provide valuable information about this entity, which could open perspectives regarding novel therapeutic approaches for children with BCP-ALL iAMP21.

https://doi.org/10.1016/j.htct.2022.09.581

CLINICAL AND MOLECULAR REPORT OF A RARE RAM POSITIVE NON-DOWN SYNDROME INFANT CBFA2T3-GLIS2 NEGATIVE, PRESENTING THE DRIVER P210-BCR-ABL1

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Objectives: Pediatric Acute Megakaryoblastic Leukemia (AMKL) comprises 4%–15% of Acute Myeloid Leukemia (AML) cases. Down Syndrome (DS)-AMKL usually displays a favorable outcome, whereas the prognosis of non-DS-AMKL is generally poor. Here we present the clinical and genomic data of a rare non-DS-AMKL infant with p210-BCR-ABL1 fusion, co-occurring with a high-risk WT1 mutation. The baby was RAM positive and CBFA2T3-GLIS2 negative, showing refractoriness to treatment. **Material and methods:** A 1-year-old girl was referred to the Hospital with a history of fever and motor developmental delay for four months. On admission, she presented ecchymosis, swelling, and organomegaly (spleen and liver). The WBC count was $26.5 \times 10^3/\mu$L, hemoglobin level was 7.6 g/dL, platelet count was $25 \times 10^3/\mu$L, and LDH was 2.887. At diagnosis, she showed bilateral myeloid sarcoma. Morphological analysis of the Bone Marrow (BM) showed clustered cytoplasmic blebs. Flow cytometry analysis revealed 83% of blast cells and a profile compatible with AMKL and RAM phenotype. The patient was treated under the high-risk AML-BFM-2004. Imatinib was incorporated into the treatment after BCR-ABL1 detection. However, minimal residual disease remained positive throughout the treatment, making BM transplantation unfeasible. Disease refractoriness was persistent even with the administration of Dasatinib, FLAG-IDA and Venetoclax. The patient died one year after diagnosis due to sepsis during BM aplasia. We performed G-banding, FISH, and Multiplex RT-PCR experiments. The molecular response was assessed by absolute quantification via RT-qPCR assays. Sanger sequencing and NGS were performed on a targeted approach. **Results:** The karyotype was 46,XX,t(9;22)(q21;q34). We observed polyploidy (3–20N). FISH in polyploid Nuclei (8N) showed four BCR-ABL1 fusion signals. In diploid metaphases, we observed two BCR-ABL1 signals. Multiplex RT-PCR detected the p210-*BCR-ABL1*. RT-qPCR monitoring detected persistent high levels of BCR-ABL1 transcripts associated with disease refractoriness. Sanger sequencing showed no GATA1 mutation. NGS revealed a missense WT1 pathogenic mutation, and CBFA2T3-GLIS2 was not present. **Discussion:** RAM phenotype is characterized by overexpression of CD56 and under-expression of CD38, CD45, and HLA-DR. This phenotype is characteristic of children, usually associated with AML-M7 (38%) and intermediate-risk cytogenetics not associated with prognostic subgroups. RAM is related to a poor prognosis with high MRD and induction failure rates compared to non-RAM patients. This phenotype is associated with the CBFA2T3-GLIS2 fusion, which is related to

extramedullary involvement, a particular blast morphology, and a worse outcome as compared to fusion-negative AML pediatric patients, including a high frequency of disease refractoriness and relapse. NGS studies did not show CBFA2T3-GLIS2 in our patient. Thus, our results indicate that RAM+ associated with the driver p210-BCR-ABL1, even with specific targeted therapy, may lead to treatment resistance regardless of CBFA2T3-GLIS2+. **Conclusion:** Here we highlight for the first time the presence of p210-BCR-ABL1 associated with RAM phenotype in the absence of CBFA2T3-GLIS2 and disease refractoriness in a non-DS-AMKL infant.

https://doi.org/10.1016/j.htct.2022.09.582

MOLECULAR CYTOGENETIC AND NEXT-GENERATION SEQUENCING STUDIES OF AN ADOLESCENT WITH ACUTE MYELOID LEUKEMIA AND INV(16) ASSOCIATED WITH A KIT PATHOGENIC MUTATION

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Objectives: Inv(16)(p13q22)/CBFb-MYH11 in Acute Myeloid Leukemia (AML) is hard to characterize and comprises about 5%–7% of all cases and 5%–9% in children. Inv(16) is associated with a good prognosis both in adults and children. However, approximately 30%–40% of affected patients experience relapse. In this context, additional type I and II mutations have been related to inv(16). KIT mutations associated with inv(16) have been related to a worse prognosis. Here we describe the clinical and molecular data of a pediatric patient with AML, presenting inv(16) associated with a KIT pathogenic mutation. **Material and methods:** A 12-year-old boy with bleeding was referred to Pedro Ernesto University Hospital (HUPE), Rio de Janeiro, Brazil. The patient presented with suspected hyperleukocyte acute leukemia. Laboratory tests revealed a WBC count of 80×10^3, a platelet count of 9×10^3, and a hemoglobin level of 5.1 g/dL. Flow cytometry results demonstrated an immunophenotypic profile characteristic of LMA-M4. We conducted G-banding, FISH, and NGS studies. **Results:** Cytogenetic analysis was non-informative. Based on the clinical and immunophenotypic data, we conducted an experimental design using PML-RARA and CBFB-MYH11 FISH probes. The patient was negative for the PML-RARA fusion, but CBFb-MYH11 was confirmed. The NGS Assay revealed a pathogenic missense mutation in KIT. The results were negative for FLT3/ITD mutations. **Discussion:** AML patients presenting inv(16)/CBFb-MYH11 usually have a good prognosis with better remission and overall survival rates compared to those with normal karyotypes. However, due to the high rates of disease relapse, the prognostic value of alterations associated with inv(16)/CBFb-MYH11 has been widely discussed in the literature. Our patient presented with excessive bleeding and heavy hemorrhage, which initially raised the suspicion of acute promyelocytic leukemia. Due to the heterogeneity of the CBFb-MYH11 fusion, featuring distinct clinical and genetic profiles depending on its molecular size molecular and the association of these profiles with specific genomic alterations, a precise genomic characterization is necessary. KIT mutations, such as the one observed in our patient, are present as alterations associated with inv(16) in approximately 9%–53% of AML cases. Previous studies have demonstrated the potential prognostic impact of KIT mutations in the affected patients. In this context, missense mutations may lead KIT to an overactive state. This type of mutation has been shown to play a pivotal role in mediating clinical response and disease refractoriness in patients treated with targeted therapeutics. Therefore, the information provided through the NGS can be crucial for treatment adaptation. **Conclusion:** In this work, we contribute with the literature showing the clinical, molecular, and genomic data of an adolescent with AML and inv(16) associated with a KIT pathogenic mutation. Besides, we highlight the importance of NGS studies in characterizing the genomic profile of patients affected by this neoplasm. Although, the prognostic implications of these findings remain to be investigated, especially in the context of new generation tyrosine kinase inhibitors targeting KIT in future clinical trials for inv(16)/CBFb-MYH11 pediatric patients.

https://doi.org/10.1016/j.htct.2022.09.583

NEXT-GENERATION GENOMIC MONITORING REVEAL A NOVEL RUNX1 PATHOGENIC MUTATION ASSOCIATED WITH TRISOMY 4 AS A SOLE ABNORMALITY IN A YOUNG ADULT WITH ACUTE MYELOID LEUKEMIA

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