Referências:

1. Stevens DL, Bryant AE. Necrotizing soft-tissue infections. N Engl J Med. 2017;377:2253-65. doi:10.1056/NEJMra1600673.
2. Bucca K, Spencer R, Orford N, et al. Early diagnosis and treatment of necrotizing fasciitis can improve survival: an observational intensive care unit cohort study. ANZ J Surg. 2013;83(5):365-70. doi:10.1111/ans.12056.
3. Eke N. Fournier's gangrene: a review of 1726 cases. Br J Surg. 2000;87(6):718-28. doi:10.1046/j.1365-2168.2000.01497.x. PMID:10848848.

https://doi.org/10.1016/j.htct.2025.104545

ID - 1870

GENOMIC INSTABILITY IN AML: ASSOCIATION BETWEEN FLT3-ITD AND CHROMOSOMAL TRANSLOCATION

CL Araújo [a], CB Cavalcante [a], RD Dias [a], RT Oliveira [a], L Sampaio [a], JV Goes [a], M Parente [a], STO Saad [b], SMM Magalhães [a], RF Pinheiro [a]

[a] *Laboratory of Cancer Cytogenomics Center for Research and Drug Development, Núcleo de Pesquisa e Desenvolvimento de Medicamentos (NPDM), Universidade Federal do Ceará (UFC), Fortaleza, Brazil*
[b] *Centro de Hematologia e Hemoterapia (Hemocentro), Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil*

Introduction: Myelodysplastic Neoplasms (MDS) and Acute Myeloid Leukemia (AML) represent spectra of a common clonal hematologic process, whose progression is often associated with genomic instability and the acquisition of somatic mutations in genes that regulate hematopoiesis. Among these alterations, the FLT3-ITD mutation stands out as a significant molecular marker of poor prognosis, whose early detection may aid in risk stratification and monitoring of clonal evolution. **Objectives:** This study aimed to investigate the presence of the FLT3-ITD mutation in patients with MDS and AML, with emphasis on karyotype analysis and mutation confirmation via Sanger sequencing. **Material and methods:** A total of 45 bone marrow samples were analyzed from patients diagnosed with MDS (subtypes EB1 and EB2) and AML (de novo and secondary), collected in EDTA tubes. Genomic DNA was extracted using the PureLink® kit (Invitrogen) and subjected to amplification of the ITD region of the FLT3 gene by end-point PCR using primers described in the literature. PCR products were visualized by 2% agarose gel electrophoresis, with preliminary detection of the mutation based on band patterns suggestive of insertions. Samples with a suspected ITD pattern were purified and subjected to Sanger sequencing, with results analyzed via electropherograms and alignment to the reference sequence from NCBI using BLAST. In parallel, conventional cytogenetic analysis was performed on bone marrow using G-banding, with at least 20 metaphases evaluated per sample. **Results:** Among the 45 patients analyzed, a 33-year-old female patient with de novo AML presented with an abnormal karyotype, showing a chromosomal translocation between chromosomes 2 and 11 [t(2;11)], a non-recurrent alteration associated with genomic instability. The FLT3-ITD mutation was initially suggested by gel electrophoresis, with a band pattern indicative of a mutated fragment. Sanger sequencing confirmed the mutation, revealing a 27-base pair insertion in the region between exons 14 and 15 of the FLT3 gene, within the juxtamembrane domain, with 94% identity to the BLAST reference sequence. **Discussion and conclusion:** The simultaneous presence of a structural chromosomal abnormality and an activating FLT3 mutation reinforces the role of genomic instability in the progression of myeloid neoplasms. Moreover, the study demonstrated the effectiveness of Sanger sequencing as the gold standard method for confirming ITD mutations, particularly in scenarios where the size and exact location of the duplication directly impact clinical interpretation. The association between altered karyotype and point mutations highlights the importance of integrating classical cytogenetics and molecular analysis in the diagnosis and prognosis of hematologic malignancies.

https://doi.org/10.1016/j.htct.2025.104546

ID - 2973

GEOSPATIAL ANALYSIS OF GENE FUSIONS IN PEDIATRIC ALL AND THEIR POSSIBLE ASSOCIATION WITH ENVIRONMENTAL FACTORS IN THE AMAZON REGION (PARÁ, 2023–2025)

YS Santos [a], KB Rodrigues [a], JCS Oliveira [a], MB Oliveira [a], MM Bernardes [b], ÁTM Tavares [a], LC Pantoja [b], AV Wanderley [b], BCM Khayat [a], AS Khayat [a]

[a] *Universidade Federal do Pará (UFPA), Belém, Brazil*
[b] *Hospital Oncológico Infantil Octávio Lobo (HOIOL), Belém, Brazil*

Introduction: Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer, accounting for 25% to 30% of pediatric neoplasms. The disease is characterized by chromosomal translocations and aneuploidies, genetic alterations that determine prognosis and treatment response. In the state of Pará, an estimated 360 new cases of leukemia are diagnosed annually, an incidence that may be influenced by regional genetic and environmental factors. **Objectives:** To investigate the potential influence of regional environmental factors on the distribution of gene fusions in patients diagnosed with ALL in the state of Pará between 2023 and 2025, considering the geographic origin of each case. **Material and methods:** This study (Ethics Committee approval No. 4.040.805) analyzed patients with ALL diagnosed at the Octávio Lobo Children's Cancer Hospital (HOIOL) between 2023 and 2025. From blood samples, RNA was extracted, converted to cDNA, and used to detect five specific gene fusions (*BCR::ABL*, *TCF3::PBX1*, *KMT2A::AFF1*, *ETV6::RUNX1*, and *SIL::*

TAL) via Nested PCR. The products were visualized by electrophoresis, confirmed by Sanger sequencing, and the data were descriptively analyzed using R Studio 12.1 Software. **Results:** Between 2023 and 2025, a survey at HOIOL identified 220 patients diagnosed with acute lymphoblastic leukemia (ALL). Of these, 56 had a detectable molecular biomarker, although residency data were available for only 41 of these patients. These patients were distributed across 22 municipalities in Pará and the city of Macapá, in Amapá. The most prevalent fusions were *ETV6::RUNX1* (61% of cases) and *TCF3::PBX1* (23% of cases). The city of Belém had the highest concentration of cases, with varied fusions: *ETV6::RUNX1* (7), *BCR::ABL* (1), and *TCF3::PBX1* (1). The municipalities of Ananindeua, Santo Antônio do Tauá, and São Félix do Xingu each presented cases of *TCF3::PBX1* (1) and *ETV6::RUNX1* (1), while Barcarena recorded *BCR::ABL* (1) and *TCF3::PBX1* (1) fusions. Cases exclusively involving the *ETV6::RUNX1* fusion were identified in Abaetetuba (2), Afuá (1), Ajuruteua (1), Canaã dos Carajás (1), Paragominas (1), Parauapebas (1), Porto de Moz (1), Viseu (1), and Macapá (4). In turn, single occurrences of the TCF3::PBX1 fusion were observed in Cametá (2), Bragança (1), Novo Repartimento (1), and Portel (1). Additionally, Capanema and Marabá each presented one case with the *SIL::TAL* fusion, and finally, the city of Moju recorded a single case with the *KMT2A::AFF1* fusion. **Discussion and conclusion:** Notably, the southeastern region of Pará (Parauapebas, Canaã dos Carajás, Marabá, Novo Repartimento, São Félix do Xingu), an area of intense mining activity, accounted for 17% of cases with poor-prognosis fusions. The recurrence of these alterations raises the hypothesis of an association with environmental exposure to heavy metals, although a direct causal relationship cannot be established. Similarly, the northeastern region of Pará (Abaetetuba, Moju, and Cametá), characterized by intensive agricultural activity, presented 9% of cases with poor-prognosis fusions, suggesting a possible link to pesticide exposure, though a causal link has not been proven. Belém, in turn, had the highest number of cases, predominantly of good prognosis, a fact that may be attributed to its large population and proximity to reference centers, which facilitates detection. The occurrence of severe leukemia in Pará, in mining and agribusiness areas, suggests a strong link with environmental factors and demands further investigation.

https://doi.org/10.1016/j.htct.2025.104547

ID - 1193

GERMLINE PREDISPOSITION TO MYELOID MALIGNANCIES: A COMPREHENSIVE ANALYSIS OF CEBPA, DDX41 AND RUNX1

D Kusma-Wosniaki [a], B Nichele-Kusma [a], H Kaur [b], E Batista-Mendes [a], M Nóbrega-Aoki [a], DL Zanette [a], JM Capo-Chichi [b]

[a] *Laboratory for Applied Science and Technology in Health, Instituto Carlos Chagas (ICC), Fundação Oswaldo Cruz (Fiocruz), Curitiba, Brazil*
[b] *Division of Clinical Laboratory Genetics, Laboratory Medicine Program, University Health Network, Toronto, Canada*

Introduction: Myeloid malignancies such as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are typically sporadic, but germline predisposition involving genes like CEBPA, DDX41, and RUNX1 is increasingly recognized. However, the interplay between germline and somatic events in these genes remains incompletely understood. **Objectives:** To investigate the prevalence, co-variant patterns, and clonality of pathogenic and likely pathogenic variants in CEBPA, DDX41, and RUNX1 in patients with myeloid malignancies. **Material and methods:** We analyzed 2,437 patients diagnosed with AML, MDS, MDS/AML, or myeloproliferative neoplasms (MPN). DNA from peripheral blood or bone marrow was sequenced using targeted NGS, covering 63 genes. Variants were processed using validated bioinformatic pipelines and classified per CAP/AMP/ASCO guidelines. **Results:** Among the cohort, AMP/ASCO Tier-1 and Tier-2 variants were most frequently detected in RUNX1 (9.97%, n = 243), followed by CEBPA (3.94%, n = 96) and DDX41 (2.63%, n = 64). RUNX1 mutations were commonly associated with co-occurring variants in ASXL1 and SRSF2, particularly in cases with double RUNX1 hits (37.5%). VAF distribution in double-mutated RUNX1 (RUNX1 highest hit: minimum: 10.9, maximum: 47.2, median: 38.5) with variants in ASXL1 (ASXL1 highest hit: min.: 4.3, max.: 49.5, median: 32.6 and/or SRSF2 (min.: 37.5, max.: 49.5, median: 46.1) were frequently consistent suggestive of a clonal process. CEBPA alterations with bi-allelic CEBPA variants (bZIP domain in-frame variant + an N-terminal loss-of-function variant). Co-occurring variants in GATA2 and WT1 were enriched in bi-allelic cases (60%) and were often seen at similar VAF (CEBPA bi-allelic highest hit: min.: 6, max.: 59.2, median: 41.9, GATA2 highest hit: min.: 5, max.: 50, median: 42,6; WT1 highest hit: min.: 5, max.: 94.1, median: 46.8), supporting a shared clonal origin and suggesting a distinct molecular signature potentially driving leukemogenesis. DDX41 variants showed a bimodal VAF distribution in double-mutated cases, with clusters likely suggestive of acquired (min.: 3, max.: 47, median: 6) or inherited (min.: 7, max.: 93, median: 49) variants. Unlike CEBPA and RUNX1, DDX41 variants were not associated with consistent co-variant patterns, suggestive of a different pathobiology. Of note biallelic disruptive DDX41 variants have been associated with hematologic malignancies with unique AML/MDS like features. **Discussion and conclusion:** Our findings reveal distinct mutational patterns in CEBPA, DDX41, and RUNX1, genes linked to hereditary hematologic cancers. Bi-allelic CEBPA mutations, formed a molecularly coherent subgroup frequently co-mutated with GATA2 and WT1 at similar VAFs, suggesting a shared clonal origin. Similarly, RUNX1 variants often co-occurred with ASXL1 and SRSF2 mutations, also showing consistent VAFs, though RUNX1 mutations were distributed across the gene. In contrast, DDX41 cases lacked co-variant patterns but exhibited a bimodal VAF distribution, with higher VAFs (>35%) suggestive of germline variants and lower VAFs (<20%) likely representing secondary somatic events. This study highlights the distinct biological and clinical profiles of germline and somatic variants in CEBPA, DDX41, and RUNX1, underscoring the need for further research into germline predisposition and its role in the pathogenesis of myeloid neoplasms.

https://doi.org/10.1016/j.htct.2025.104548