complementary DNA synthesis, conventional polymerase chain reaction (PCR), and genetic sequencing. Clinical, epidemiological, and laboratory data were analyzed from physical and electronic records of the HEMOAM, with statistical analyses performed to describe and determine the findings. Pathogenic variants were identified from the obtained sequences, also analyzed the Variant Allele Frequency (VAF) and classified according to CancerVar. **Results:** The study population present a higher prevalence of females, 11 (61%), an average age of 50 years (± 23.55). Regarding laboratory data, patients presented with erythropenia, thrombocytopenia, and leukocytosis, with counts of 2,637 × 10/mm^3, 43,500/mm^3, and 45,346/mm^3, respectively. The classical variant (type-A), c.864_867dupTCTG (W288Cfs), was identified in 3 patients (16%), while the pathogenic c.385C>G (E129Q) variant in 18 (100%). Curiously, 11 patients (11/18) carrier the E129Q variant, and 3 (3/11) carrier both W288Cfs and E129Q variants, which led to death outcomes. Furthermore, these patients demonstrated a Variant Allele Frequency (VAF) exceeding 40%, with a mean VAF of 44% [41% - 61%] and 43% [40% - 47%], respectively. **Discussion:** In this study, a low frequency of the W288Cfs variant was observed, which differs from other studies. However, the VAF results are consistent with studies highlighting that patient with a burden exceeding 40% for the NPM1, indicate high allele load, may contribute to the onset of leukemogenesis. Furthermore, a high VAF may indicate resistance to chemotherapy treatment, influencing lower patient survival rates. **Conclusion:** Pathogenic variants and increased allelic load in the NPM1 were frequently observed in AML patients, possibly related to hematological alterations. Our study highlights the importance of variant analysis in the coding region of *NPM1* through genetic sequencing, evaluation of mutational allelic burden quantification of NPM1 variants at diagnosis is essential to define the risk stratification profile and providing information for therapeutic approaches in disease treatment.

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PATOGÊNESE E TRATAMENTO DA LEUCEMIA MIELOIDE AGUDA SECUNDÁRIA

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Introdução/Objetivos: A leucemia mieloide aguda secundária (LMA-2ª) está associada a um prognóstico ruim, com taxa de resposta clínica e sobrevida inferior em comparação à leucemia mieloide aguda primária (LMA-1ª). A LMA-2ª surge a partir de eventos epigenéticos causados por quimioterapia citotóxica, ou por distúrbios mielodisplásicos ou mieloproliferação. Clones leucêmicos são frequentemente quimiorresistentes, com mutações diferentes de acordo com sua etiologia, o que clama por terapias especificas para LMA-2ª. O presente trabalho tem como objetivo explorar a patogênese e os tratamentos disponíveis para LMA-2ª. **Materiais e métodos:** Foi realizada uma revisão de literatura sobre o tema na base de dados PUBMED a partir de janeiro de 2020. Foram utilizados os descritores *Pathogenesis OR Treatment AND Secondary acute myeloid leukemia*. A busca resultou em 1892 artigos. Foram excluídos artigos de opinião, relatos ou series de casos. Foram incluídos 27 artigos que abordaram a patogênese e o tratamento para LMA-2ª associado a desfechos clínicos. **Discussão/Resultados:** Em relação à patogênese, mutações como JAK2 V617F e BCR-ABL se refletem em alterações no TP53, RUNX1, SETBP1, reguladores epigenéticos e genes do spliceossomo. Essas alterações, junto com mudanças no microambiente da medula óssea, como neoangiogênese, alterações pró-inflamatórias e fibrose, causam instabilidade genômica que pode resultar em LMA-2ª. Além disso, quimioterapia com agentes alquilantes forma ligações cruzadas no DNA, provocando quebra das fitas no processo de reparo, enquanto os inibidores da topoisomerase II provocam translocações cromossômicas, como KMT2A em 11q23 e RUNX1 em 21q22. Essas aberrações genéticas em células-tronco hematopoiéticas conferem proliferação celular e sobrevida clonal, o que, eventualmente, pode ocasionar a emergência da LMA-2ª. O seu tratamento varia conforme a condição do paciente. Para aqueles que não possuem comorbidades associadas, a quimioterapia intensiva com citarabina e daunorrubicina tem sido a abordagem usual, com taxas de resposta clínica e sobrevida global em torno de 56%. No entanto, essa opção apresenta riscos, como toxicidade hematológica e infecções. Para os pacientes com comorbidades, a quimioterapia não intensiva, utilizando agentes hipometilantes, está relacionada com taxas de resposta baixa, cerca de 30%, porém com melhora na qualidade de vida. O transplante de células-tronco hematopoiéticas (alo-HTC) é uma alternativa para aqueles que respondem ao tratamento inicial, com taxa de sobrevida global ao redor de 46%, muito embora envolva maiores riscos, como mortalidade relacionada ao tratamento e doença enxerto versus hospedeiro. Além disso, terapias alvo e imunoterapia, tais como inibidores de IDH e FLT3, têm sido boas opções para pacientes com mutações específicas, apesar de ainda existirem importantes efeitos colaterais. **Conclusão:** A melhor estratégia terapêutica seria atingir a remissão completa seguida de alo-HTC. No entanto, a idade avançada, comorbidades e menor taxa de remissão limitam essa abordagem ideal a bem poucos pacientes. Deste modo, faz-se necessário que novas opções terapêuticas sejam investigadas, de forma que os pacientes acometidos pela LMA-2° tenham um melhor prognostico.

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MULTIPLEX PCR FOR DETECTION OF NPM1 W288CFS AND FLT3-ITD MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS IN AMAZONAS STATES, BRAZIL

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Introduction: Acute Myeloid Leukemia (AML) is a hematological disease clonal characterized by proliferation neoplasm cells in the bone marrow and peripheral blood, in consequence genetic alterations at hematopoietic stem cells. Genetic variants, as *FLT3-ITD* and *W288Cfs NPM1* are common in the AML, these variants are important in the diagnosis and treatment of the disease. **Objective:** In this study, we developed a multiplex PCR method to detection of *NPM1 W288Cfs* and *FLT3-ITD* variants in AML patients. **Materials and methods:** Bone marrow sample was collected from AML patients. The multiplex PCR was performed using specific primers for *NPM1 W288Cfs* and *FLT3-ITD* variants, the amplified products were separated by electrophoresis with a 2.5% agarose gel. Clinical, epidemiological, and laboratory data were analyzed from physical and electronic records, with statistical analyses performed to describe and determine the findings. The European LeukemiaNet (ELN) risk stratification was used, considering molecular results of the FLT3-ITD and *NPM1 W288Cfs*. **Results:** The study population was composed of 40 AML patients, female patients were most frequent with 25 (62.5%), the average age was 52 years ± 21.46. The study included 30 (75%) primary AML, 6 (15%) secondary and 4 (10%) relapsed cases, M1 and M2 subtype were prevalent, 30% and 25%, respectively. Hematological data revealed that the patients present anemia 2,728 × 10^3/mm^3 (± 0,572), thrombocytopenia 50,28/mm^3 (43,37) and leukocytosis 57,40/mm^3 (± 111,2). Curiously, only 4 (10%) patients were NPM1 W288Cfs positive. Accord ELN risk stratification, 3 (7.5%) patients were classified as favorable (NPM1+ e FLT3-ITD-), 30 (75%) adverse (NPM1-) and 7 (17.5%) as intermediary (NPM1+ e FLT3-ITD+ / NPM1- e FLT3-ITD+). **Discussion:** *NPM1 W288Cfs* variant present low frequency (10%), contrary to other studies, while the risk stratification data are consistent with the ELN. The current World Health Organization classification-2022 highlights the need to implement genetic and molecular tests as routine to evaluate AML patients. Thus, the techniques used had a high sensitivity and specificity in identifying the FLT3-ITD and *NPM1 W288Cfs* variants, since allowed to characterize AML patients and defining genetic abnormality. **Conclusion:** Here, we observed a frequency low of the *NPM1 W288Cfs* variant in the Amazonas State. The multiplex PCR consist of assay in-house of low cost, with good sensitivity and specificity for FLT3-ITD and *NPM1 W288Cfs* mutations, allowing to detect the mutations simultaneously.

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PATHOGENIC MISSENSE VARIANTS IN THE TP53 IN ACUTE MYELOID LEUKEMIA PATIENTS TREATED AT A REFERRAL HOSPITAL IN THE AMAZONAS STATE, BRAZIL

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Introduction: Acute Myeloid Leukemia (AML) is a malignant clonal hematological neoplasm characterized by the infiltration of blasts, resulting in the failure of mature blood cells in the bone marrow and peripheral blood. Different genetic alterations have been reported in AML. Mutations in the *TP53* gene are associated with adverse prognosis in AML, leading to reduced patient survival. **Objectives:** In this study, we investigated variants in the coding region of the *TP53* gene in AML patients in Amazonas, Brazil, focusing on clinical epidemiological aspects and pathogenicity. **Materials and methods:** Bone marrow sample was collected from AML patients. The RNA extraction and complementary DNA synthesis were performed. The *TP53* exons 2 to 11 were amplified, and nucleotide sequencing was performed by the Sanger method. Hematological data from the patients were also evaluated using peripheral blood. **Results:** The study included 25 patients diagnosed with AML, 19 cases de novo, 4 secondary and 2 relapsed AML. Here, 17 (68%) patients were female, the average age was 50.20 years ± 18.30. Hematological parameters showed erythropenia with a red blood cell count of 2.89 (2.355-3.245), thrombocytopenia with 20,000 (13,500-63,000), and leukocytosis with 14,000 (4,385-29,120). Pathogenic variants were predominantly identified in exon 4, also was found in the exons 3, 5, 7, 8, 10 and 11. A total of 47 variants was identified at 25 patients, including multiple hits. Nine variants were classified by CancerVar as pathogenic (L32Q, M43K, A119D, D147N, D181N, R248Q, E271D, P278L and K372N), six as uncertain significance (N29D, Q38H, P72R, W91G, R213R and K357M), as benign (F52V), and 4 variants were not found in the database (L44M, Q51Pfs, P79T and L129Ifs). Additionally, 2 silent variants and 3 frameshift variants were identified. **Discussion:** AML patients commonly present hematological parameters such as erythropenia, thrombocytopenia, and leukocytosis, corroborating with study. The *TP53* gene is frequently mutated in human cancers and present in AML cases (10-20%). In this study, least one alteration was identified in the 25 patients. Variants are prevalent in the DBD region and corroborates with the study findings. Mutations in the DBD region can confer loss of function (LOF), attributing a deleterious gain of function (GOF) and rendering resistance to standard AML treatment. **Conclusion:** The present study presents results that diverge from the current literature in several significant aspects, contradicting previously established data. The *TP53* mutations were prevalent in the AML patients from Amazonas state. These findings