benignas/provavelmente benignas, 58% como VUS ou não previamente descritas, e 0,5% como patogênicas/provavelmente patogênicas. Em média, 36 variantes foram detectadas por amostra, e variantes potencialmente relevantes foram observadas nos genes: VHL, EPAS1, HBB, HSF1 e HIF3A, incluindo 6 variantes novas. A variante patogênica encontrada em VHL foi confirmada como germinativa após análise de amostra de swab oral do mesmo paciente por sequenciamento de Sanger. O diagnóstico da eritrocitose representa um desafio devido às características clínicas associadas a diferentes etiologias. Neste trabalho, descrevemos características clínicas e moleculares de pacientes com PV, ESA e EI. Seis variantes novas e uma variante patogênica/provavelmente patogênica foram identificadas. O painel customizado de NGS desenvolvido surge como uma ferramenta valiosa para identificar variantes genéticas subjacentes à eritrocitose.

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CHALLENGES IN INTERPRETING AVWS SCREENING IN POLYCYTHEMIA VERA: CORRELATIONS WITH CLINICAL AND LABORATORY FEATURES

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Background: The risk of thrombotic and hemorrhagic complications frequently coexists in myeloproliferative neoplasms (MPN), partly due to the occurrence of acquired von Willebrand syndrome (AVWS), particularly in essential thrombocythemia. However, the value of screening for AVWS in other MPNs is not so clear. Additionally, it is unknown whether laboratory-diagnosed AVWS correlates with clinical manifestations. Therefore, we aimed to investigate the connection between specific clinical and laboratory features and AVWS, and to determine if AVWS was associated with bleeding in our polycythemia vera (PV) cohort. **Methods:** This is a cross-sectional study involving adult PV patients followed at Hospital das Clínicas of the Faculty of Medicine, University of São Paulo, Brazil. Screening for AVWS was conducted using von Willebrand factor antigen (VWF Ag) and activity levels measured by the ristocetin cofactor assay (RCO). AVWS was defined as a VWF:RCo/Ag ratio < 0.6. Additionally, we collected data on thrombotic and bleeding complications. **Results:** We evaluated 46 of 597 patients (54% male, median age 62.5 years) diagnosed with polycythemia vera who were screened for AVWS from 2012 until 2023. In only three patients, the tests were ordered immediately after a bleeding event. At the moment of the screening, the median blood counts were: hematocrit 43% (IQR 38-51), leukocytes 11.7×10^9/L (8.0-16.8), platelets 572×10^9/L (IQR 307-952). Median VWF antigen was 146% (range 64-464), median VWF:RCo was 58% (range 1-159) and median function/antigen ratio (VWF:RCo/Ag) was 0.42 (range 0.01-0.78). Thirty-six patients (78%) had a screening test (VWF:RCO / Ag < 0.6) positive for AVWS, and this parameter was correlated to thrombosis ($p = 0.03$), but not to bleeding ($p = 0.58$). There were 12 bleeding events (26% of the cohort) and neither VWF antigen, VWF:RCo, hematocrit, leukocyte count, platelet count or risk group could be associated with these events. It was observed that VWF antigen was higher with higher platelet counts ($p = 0.02$). A reduced function/antigen ratio (VWF:RCo/Ag) showed a significant association with leukocytosis and thrombocytosis, but not with hematocrit above normal range. In nine patients, the AVWS screening tests were done on at least two occasions. We observed that the level of VWF:RCo increases when the patients achieve the target hematocrit level below 45%. However, the VWF:RCo/VWF ratio improved only when the platelets levels lowered after cytoreduction. **Conclusions:** : Most of the patients in our cohort (78%) had a positive screening for AVWS by the VWF:RCO / Ag ratio criteria. However, we did not show a correlation with bleeding events or blood count parameters. Despite the significant number of bleeding events, establishing the diagnosis of AVWS or a correlation with bleeding events is a challenge for patients with PV.

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INTERFERENCE OF BCR::ABL1 SPLICING VARIANTS IN MANAGEMENT OF CHRONIC MYELOID LEUKEMIA PATIENTS

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Introduction: Chronic Myeloid Leukemia (CML) represents a paradigm in oncology, characterized by the hallmark Philadelphia chromosome resulting from the t(9;22) reciprocal translocation. This abnormal chromosome gives rise to the *BCR::ABL1* fusion gene, which encodes a constitutively active tyrosine kinase protein driving leukemogenesis. The use of tyrosine kinase inhibitors (TKI) have revolutionized CML treatment, demonstrating remarkable efficacy. However, TKI resistance, primarily attributed to point mutations within the *BCR::ABL1* kinase domain (KD) remains a significant clinical challenge, affecting approximately 20-30% of patients. The gold standard technique for monitoring mensurable residual disease in CML is the quantitative PCR (qPCR), while Sanger sequencing is utilized to identify KD mutations upon relapse. **Objective:** This study aims to address the specific challenges posed by two *BCR::ABL1* splicing variants — the deletion of exon 7 (Δ7) and the insertion of 35 base pairs between exons 8 and 9 (INS35) — in the molecular monitoring of CML patients. These splicing events occur frequently in CML, observed in approximately 20-25% of cases. Concurrent occurrence of alternative splicing variants with point mutations complicates the detection of low-frequency mutations, potentially leading to mislead or misinterpret

results. Additionally, splicing variants are detected by qPCR and incorporated into the Molecular Response (MR) calculation, which may result in poorer MR outcomes, as literature suggests these variants exhibit reduced sensitivity to TKIs. **Methods:** To address these challenges in CML management, we developed a PCR-based technique coupled with fragment analysis to accurately identify these variants prior to sequencing. We validated this test with 30 samples with Δ7 and/or INS35, prior identified by the Sanger sequence. Moreover, we designed a specific qPCR assay to quantify splicing variants exclusively. **Results and conclusion:** We performed in silico modeling of the wild-type versus Δ7 and INS35 *BCR::ABL1* isoforms, showing the KD disruption caused by the early stop codon resulting from the frameshift caused by Δ7 and INS35. Additionally, our findings indicate that this molecular approach enables highly accurate and sensitive identification of splicing variants, even at very low levels. We observed that these events are independent of CML status, also occurring in healthy donor samples. Furthermore, Δ7 and INS35 are present throughout the longitudinal patient follow-up and are not associated with TKI failure. However, Δ7 with a variant allele frequency (VAF) greater than 0.15 is more prevalent in samples with higher *BCR::ABL1* transcript counts, whether at diagnosis, failure, or relapse. This study highlights how Δ7 and INS35 can confound mutational testing results and demonstrates how to overcome these challenges for a more precise analysis of point mutations in CML patients carrying splicing variants.

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PHILADELPHIA CHROMOSOME-NEGATIVE CHRONIC MYELOID LEUKEMIA WITH BCR::ABL1 FUSION

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Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the chromosome philadelphia (Ph) in 90-95% of cases. The Ph chromosome results from the reciprocal t(9;22)(q34.1;q11.2) and encodes the fusion gene *BCR::ABL1*. The chimeric tyrosine kinase oncoprotein is constitutively activated, leading to continuous cell proliferation and driving leukemogenesis. **Case report:** Herein, we present a rare case of a 36-years-old male patient diagnosed with Ph-negative CML. The patient was admitted to the Hematology Department of Instituto Nacional de Câncer (INCA) in September 2019 presenting persistent fever, splenomegaly, weight loss and night sweats. Blood workup showed significant leukocytosis (229.450/μL), thrombocytosis (639.000/μL) and eosinophilia (16.000/μL, 7%). At diagnosis, the patient exhibited 5% of myeloid blasts in blood and the b2a2 isoform of *BCR::ABL1* was detected by qualitative reverse-transcription polymerase chain reaction (RT-PCR). The patient was classified as high risk according to the Sokal Index (2.1) and his initial treatment with hydroxyurea was followed by Imatinib (600 mg/day). Molecular monitoring was performed by qualitative RT-PCR for p210, because *ABL* control gene failed to amplify in our quantitative RT-PCR (qPCR) assay in several samples, despite the successful amplification of *BCR::ABL1* fusion gene. After 18 months, and again after 42 months, clinical worsening due to the patient's low adherence to treatment led to a change in therapy to Nilotinib (800 mg/dia) and Dasatinib (100 mg/dia), respectively. In 2023, the patient was hospitalized with accelerated phase CML (AP-CML) characterized by basophilia and no detectable myeloid blast. Conventional cytogenetic analysis was performed in bone marrow sample from relapse and revealed a normal karyotype (46, XY[20]), however, FISH using LSI *BCR::ABL1* Dual Color/Dual Fusion Probe identified one *BCR* signal; two *ABL* signals and a single fusion signal in 86/100 nuclei analyzed, consistent with the *BCR::ABL1* detection by RT-PCR. Additionally, the F317L mutation was detected by the *BCR::ABL1* mutational testing realized at relapse. The mutation in AP-CML scenario led to the recommendation for hematopoietic stem cell transplantation. Three months post-transplantation, the patient rejected the graft, showing complete autologous recovery. Nilotinib treatment (800 mg/dia) was reintroduced with satisfactory clinical response. **Discussion:** This case describes a rare instance of CML with a normal karyotype but positive for *BCR::ABL1*, often referred to as Ph-negative CML. Literature suggests that those cases may arise from complex chromosomal abnormalities, including three-way or multi-way translocations involving chromosomes 9, 22, and additional chromosomes. In this case, the normal karyotype by conventional cytogenetics together to the FISH pattern implies the possibility of a cryptic insertion between chromosomes 9 and 22, where a partial *ABL1* segment from chromosome 9 may have inserted into the *BCR* locus on chromosome 22, thus hindering detection by conventional cytogenetics. This highlights the importance of integrating molecular cytogenetics and molecular biology techniques with conventional cytogenetics for comprehensive CML diagnosis, as cases with a normal karyotype may still harbor complex chromosomal abnormalities only detectable by FISH and PCR testing.

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DIFFERENTIAL GENE EXPRESSION AND PATHWAYS ENRICHMENT ANALYSIS IN TWO RNA-SEQ DATASETS OF JAK2V617F POSITIVE CELL LINE, TREATED WITH RUXOLITINIB, INDICATE FOCAL ADHESION PATHWAY MODULATION

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