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Evaluate how TKIs affect the population dynamics of leukemic cells during treatment according an populational decaying model, with the goal of optimizing treatment strategies for CML. Using ELN guidelines, this study modeled *BCR::ABL1* p210 decay over 12 months post-TKI treatment via logarithmic interpolation, yielding a predictive equation. Key assumptions included a closed population, non-overlapping generations, density-independent mortality, discrete time, and exponential decay. Wolfram Mathematica was used for curve fitting. This study uses ELN guidelines and logarithmic interpolation to model *BCR::ABL1* decay in CML patients undergoing TKI therapy. Starting from month 3, the equation ($y = 99.72e^{-0.766x}$), derived from RT-qPCR data, predicts *BCR::ABL1* levels with an R^2 of 0.99, indicating high accuracy. The model, reflecting the clinical timeline of *BCR::ABL1* monitoring, demonstrates the efficacy of TKI therapy and offers a reliable framework for predicting *BCR::ABL1* trajectories, aiding in treatment monitoring and decision-making. This study uses evolutionary and ecological modeling to evaluate *BCR::ABL1* decline in CML patients undergoing TKI therapy. Initial *BCR::ABL1* levels and decay rates are critical for predicting treatment success according to decaying model. This approach suggests more aggressive initial treatment for high *BCR::ABL1* levels, using potent TKIs and combination therapies. This approach aims to overcome resistance, enhance treatment efficacy, and personalize therapy based on patient-specific characteristics. This research uses evolutionary, and population decline models to study CML treatment. Initial *BCR::ABL1* levels could predict treatment response, suggesting personalized strategies could improve outcomes. Further validation is needed.

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PREDICTIVE VALUE OF QUANTITATIVE RT-QPCR BCR::ABL1 AT DIAGNOSIS AND EUTOS SCORE FOR IMATINIB RESPONSE IN CML PATIENTS

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The primary objective was to evaluate the association between the levels of *BCR::ABL1* at diagnosis (BCR0), as measured by quantitative RT-qPCR, and the EUTOS score in patients with chronic myeloid leukemia (CML), and their association with *BCR::ABL1* transcripts at 3 months after tyrosine kinase inhibitor therapy (BCRM3). We conducted a cross-sectional study evaluating 35 patients diagnosed with CML, using quantitative RT-qPCR to measure BCR0. The relationship between the EUTOS score, BCR0, and BCRM3 was evaluated using Spearman's rank correlation coefficient (Rho). Linear regression modelling was employed to examine the predictive capacity of the EUTOS score and BCR0 on BCRM3. Model fit was assessed using the adjusted coefficient of determination (R^2). Statistical significance was determined for p-values less than 0.05. A total of 35 patients with CML were analyzed, with *BCR::ABL1* activity assessed at diagnosis. Of these, 68.6% were male. The median age was 39.1 years (IQR: 23.5). The median EUTOS score was 58 (IQR: 69.5), the median percentage of blasts in the bone marrow at diagnosis was 6% (IQR: 8), and the median percentage of CD34 positive cells was 1.25% (IQR: 2). The median BCR0 level was 96% (IQR: 70.5). The correlation between BCR0 and BCRM3 was 0.63 (p-value < 0.01), demonstrating a strong positive correlation. Similarly, the correlation between EUTOS score and BCRM3 was 0.55 (p-value < 0.001), also indicating a strong positive correlation. In the linear regression analysis, using EUTOS and BCR0 as independent variables, the regression model for BCR0 had a slope coefficient of 0.05 with an R^2 of 0.6 (p-value < 0.01), indicating that BCR0 significantly predicts BCRM3. The EUTOS regression model had a slope coefficient of 0.3 with an R^2 of 0.45 (p-value < 0.01), also indicating a significant predictive value. In the multivariate model, the coefficients were 0.2 for EUTOS (p-value: 0.03) and 0.03 for BCR0 (p-value < 0.001). The multivariate model was more robust, with an R^2 of 0.7 (p-value < 0.01), indicating that the combination of EUTOS and BCR0 provides a better prediction of BCRM3. The observed median BCR0 level of 96% underscores the high disease burden at diagnosis. The correlation analysis indicated a weak, non-significant association between BCR0 and EUTOS score (Rho = 0.317, p = 0.06), suggesting that while both are prognostic markers, they may reflect different aspects of disease biology. In contrast, the strong positive correlations between BCR0 and BCRM3 (Rho = 0.63, p < 0.01) and between EUTOS score and BCRM3 (Rho = 0.55, p < 0.001) underscore their relevance in predicting early treatment response. Linear regression analysis further demonstrated the predictive value of BCR0 and EUTOS scores. The BCR0 regression model, with a slope coefficient of 0.05 and R^2 of 0.6, and the EUTOS model, with a slope coefficient of 0.3 and R^2 of 0.45, both indicated significant predictive power. Notably, the multivariate model, incorporating both BCR0 and EUTOS scores, was more robust ($R^2 = 0.7$), suggesting that a combined approach provides a superior predictive framework for assessing early response to imatinib therapy. These findings highlight the importance of integrating multiple prognostic markers to enhance predictive accuracy in CML

management. The significant associations and robust multivariate model support the use of BCR0 and EUTOS scores as complementary tools in clinical practice.

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TRANSCRIPTOMIC PROFILING SUGGESTS DYSREGULATED ACTIVATION OF B AND T LYMPHOCYTES IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Introduction and aims: Myeloproliferative neoplasms (MPN) are clonal hematological diseases characterized by exacerbated proliferation of cells belonging to the myeloid lineage. Gain of function driver mutations in the Janus Kinase 2 (JAK2), calreticulin (CALR), and thrombopoietin receptor (MPL) genes expressed in the hematopoietic stem cells are the main alterations related to MPN's pathogenesis. These mutations lead to JAK-STAT pathway overactivation, myeloproliferation, inflammation, and bone marrow fibrosis. Along with these genetic alterations, altered immune cell activation may contribute to polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (MF) pathophysiology. Potential dysregulation in the effector functions of adaptive immune cells may contribute to antitumor response escape and, therefore, favor the clonal expansion of altered myeloid cells in MPN. The present study evaluated the transcriptomic signature of B and T lymphocytes from MPN patients using *in silico* approaches. **Subjects and methods:** Public peripheral blood microarray data from PV (n = 41), ET (n = 19), MF (n = 9), and control (n = 21) samples were downloaded from the Gene Expression Omnibus (GEO) databank (#GSE26049). Using this data, we performed the Gene Set Enrichment Analysis (GSEA) to identify the immune cell signatures in PV, ET, and MF. We also determined the Differentially Expressed Genes (DEGs) between MPN patients and controls using R software to identify potential alterations in the expression of immune-related genes between MPN patients and controls. In the Human Protein Atlas website (https://www.proteinatlas.org/), we selected the option *"single cell type expression cluster (RNA)"* and *"B-cell – B-cell function", "T-cells – T-cell receptor"* to retrieve the gene signature of B and T lymphocytes. Then, we merged the gene expression of B and T cells with the DEGs retrieved from the three MPN categories to describe the signature of B and T lymphocytes in PV, ET, and MF. **Results:** GSEA revealed that T and B lymphocyte-related genes are enriched in the transcriptome of PV, ET, and MF patients. However, most of the genes associated with T and B cell activation, signaling, and surface membrane receptors were downregulated, mainly in PV and MF samples. In MF samples, GSEA revealed an exclusive positive enrichment in CD4+ T cells, while ET samples showed and unique positive enrichment of naïve and memory B cells, B cell surface receptors, and plasma cells. DEGs analysis also pointed to the downregulation of T cell-related genes compared to controls, corroborating with GSEA data and revealing the potential impairment of biological processes like lymphocyte activation, proliferation, migration, and costimulation. Key B cell-related genes like CD19, CD22, BLK, HLA-DQA1, and immunoglobulin receptors were downregulated in PV and MF DEGs compared to the controls, while the opposite profile was observed in ET samples. **Conclusion:** B and T lymphocytes from MPN patients present alterations in the expression of key genes related to cell activation, signaling, and effector functions. The gene signature of these cells seem to be different between PV, ET, and MF, suggesting that B cells are more activated in ET and CD4+ T cells in MF. Together, these data suggests that B and T lymphocytes function is altered in MPN.
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ENHANCED DETECTION AND MONITORING OF CML USING MULTIPLEX REAL-TIME PCR WITH MAGNETIC RNA EXTRACTION

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Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of a BCR-ABL1 rearrangement. Its detection and quantification by real-time quantitative PCR (RQ-PCR) play a central role in CML diagnosis, therapy monitoring, and sequencing to identify resistance mutations in case of therapy failure. As previously demonstrated (Pugliesi et al., Hematol Transfus Cell Ther. 2023;45 Suppl 4), combining magnetic RNA extraction with the use of only 2mL of peripheral blood (PB) provides a fast, cost-effective, and highly sensitive strategy for CML diagnosis and monitoring. **Objectives:** To optimize the existing method by integrating magnetic RNA extraction with multiplex real-time PCR amplification for both the BCR (internal control) and BCR::ABL1 p210 transcript within the same PCR reaction. This enhancement aims to increase scalability and traceability while reducing costs and turnaround time (TAT). **Materials and methods:** Total RNA was extracted from 100 PB samples. After erythrocyte lysis, leukocytes from 2mL of PB were automatically extracted using Extracta-MPTA (Loccus do Brasil). To validate a multiplex assay combining the BCR gene and the target BCR::ABL1, the reference standard curve ERM-AD263 was used to calibrate the assay, and a commercial reference RNA was used to set the International Scale (IS%). BCR and BCR::ABL1 were measured using previously validated singleplex assays and by multiplex (BCR and BCR::ABL1 in the same reaction). Multiplex assay accuracy was measured by comparing it to the previously validated singleplex real-time PCR for BCR and BCR::ABL1 copy number and BCR::ABL1 ratio (IS%) using 100 CML samples (paired t-test). Precision was