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

determined using internal quality control (IQC) (BCR::ABL1: 1%) in duplicates in 20 independent assays. **Results:** The singleplex and multiplex five-point standard curves for BCR and BCR::ABL1 were completely superposed (Spearman correlation, $p = 0.003$). Comparisons in 100 samples revealed that BCR and BCR::ABL1 measurements were similar (paired t-test, $p = 0.42$ and 0.07, respectively) and that BCR::ABL1 IS% values were identical ($p = 0.18$). Pairing was significantly effective for all determinations ($p < 0.0001$). The total coefficient of variation for internal quality control (IQC) was 25%, with a standard deviation of 0.36. Utilizing the multiplex test reduced the turnaround time (TAT) by 20%, allowing results to be released within 24 hours of sample reception. **Discussion:** As demonstrated, the combination of automated magnetic RNA extraction from 2mL of PB with the quantification of the BCR::ABL1 gene for patients under CML monitoring is an effective, fast, safe, and low-cost method. **Conclusion:** The integration of automated magnetic RNA extraction from 2mL of peripheral blood with multiplex real-time PCR for BCR::ABL1 quantification provides a robust, efficient, and economical approach for monitoring CML patients. This optimized method significantly reduces turnaround time, enabling results to be available within 24 hours of sample reception. Such a strategy is particularly advantageous for public healthcare systems, such as the Brazilian Unified Health System (SUS), enhancing accessibility and ensuring timely and accurate monitoring of CML patients. The streamlined process supports scalable and traceable diagnostics, ultimately contributing to improved patient management and outcomes.

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ANÁLISE DA IMUNIDADE MEDIADA POR CÉLULAS EM PACIENTES COM LEUCEMIA MIELOIDE CRÔNICA

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Introdução: A leucemia mieloide crônica (LMC) representa cerca de 15% a 20% dos casos de leucemias, acometendo principalmente o sexo masculino e a faixa etária de 40 a 60 anos. Na fase crônica (FC), os pacientes apresentam uma medula óssea hipercelular, com predomínio de granulócitos, concomitante à leucocitose com desvio à esquerda. Estes processos podem levar a exaustão ou anergia das células citotóxicas, ocasionando uma falha na defesa tumoral. Pacientes podem evoluir da FC até a fase de transformação (FT), no qual é marcada pelo aumento dos números de blastos ocupando a medula óssea. **Objetivo:** Quantificar a produção de perforina (PrF) e granzima B (GzB) por linfócitos citotóxicos após estímulo com IL-2 e IL-15 em pacientes com LMC e comparar com a fase de evolução da doença. **Materiais e métodos:** Foram avaliados seis pacientes com LMC atendidos no Hospital das Clínicas-UFTM, sendo cinco homens e uma mulher, com idades de 44 a 69 anos. Quatro foram classificados em FC e dois em FT. Células mononucleadas do sangue periférico (PBMC) foram separadas por meio do gradiente de densidade com Ficoll-Paque®. As PBMCs foram dispostas em uma placa de cultura e divididas em: não tratadas, estímulo com IL-2 e estímulo com IL-15, em triplicatas. Foram incubadas a 37°C por 72h, em meio RPMI. Após o período de incubação, as células foram marcadas com anti-CD3, anti-CD8, anti-CD56, anti-perforina clone DG9 (forma ativada), anti-perforina clone BD48 (forma inativa) e anti-granzima B. As células T CD8 foram identificadas como CD3+CD8+ e células natural killer (NK) CD3-CD56+. Foram adquiridos 1000 eventos em citômetro de fluxo e avaliado o percentual de células que expressavam PrF e GzB. Por fim, foi realizada a análise estatística dos resultados, considerando intervalo de confiança de 95%. **Resultados:** Diante dos achados, observa-se uma clara diferença no número de células que expressam proteínas citolíticas entre pacientes que estão na FC e na FT. Indivíduos na FT apresentaram menor quantidade de células TCD8 + que expressam PrF ativada comparado aos que estão na FC, tanto sem tratamento, quanto após estímulo com IL-2 ou IL-15 ($p = 0{,}061$, $p = 0{,}001$ e $p = 0{,}004$, respectivamente). No entanto, a expressão de PrF inativa nestas células não diferiu entre os grupos. Já em relação à expressão de GzB, observou-se um menor percentual de linfócitos TCD8 na FT após estímulo com IL-2 ($p = 0{,}002$) e IL-15 ($p = 0{,}038$) comparado aos que estão na FC. Considerando as células NK, pacientes na FT apresentaram um menor número de células com PrF ativada na ausência de estímulo ($p = 0{,}043$) e quando estimuladas com IL-15 ($p = 0{,}010$). Quanto ao número de NK que expressavam PrF inativa, observou-se uma diferença entre os grupos apenas quando as células foram estimuladas com IL-2 ($p = 0{,}016$), com menor expressão na FT. A quantidade de células NK que expressam GzB não diferiu entre os grupos. **Discussão:** Como a imunovigilância possui um importante papel no combate ao câncer, a menor expressão de proteínas citolíticas em pacientes na FT poderia ter desempenhado um papel na progressão da doença, propiciando a evolução de casos em FC para a FT. Outro ponto a se considerar seria a possibilidade de um consumo mais exacerbado destas proteínas, na tentativa de prestar um combate mais intenso ao aumento de blastos na FT, chegando até mesmo a um quadro de exaustão celular. **Conclusão:** Pacientes com LMC em FT apresentam menores quantidades de linfócitos citotóxicos expressando PrF e GzB comparados àqueles em FC.

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DIAGNÓSTICO SIMULTÂNEO DE TROMBOCITEMIA ESSENCIAL E MIELOMA MÚLTIPLO: UM RELATO DE CASO

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