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

https://doi.org/10.1016/j.htct.2024.09.786

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.
Funding: FAPESP grants #2019/18013-8 and #2022/13366-2.

https://doi.org/10.1016/j.htct.2024.09.787

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