IMMUNOPHENOTYPING IN THE DIAGNOSIS OF MYELOPROLIFERATIVE NEOPLASMS USING FLOW CYTOMETRY

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Abstract: *Myeloproliferative neoplasms (MPNs) are a group of chronic hematologic malignancies characterized by the clonal proliferation of myeloid precursors in the bone marrow. The timely and accurate diagnosis of these disorders is crucial for effective management, prognosis, and therapeutic decision-making. While molecular techniques and bone marrow morphology remain the gold standard, flow cytometry-based immunophenotyping is emerging as a powerful adjunctive tool that can support diagnosis, particularly in early or atypical cases.*

Objective: To evaluate the effectiveness and diagnostic utility of flow cytometric immunophenotyping in the detection and classification of MPNs, specifically essential thrombocythemia (ET), primary myelofibrosis (PMF), and polycythemia vera (PV). The goal was to identify unique

immunophenotypic patterns that could serve as reliable markers for distinguishing between these conditions.

Materials and Methods: The study included patients with a clinical and hematological suspicion of MPNs who were examined at the hematology department of [Name of Center]. Bone marrow aspirates and peripheral blood samples were collected. Flow cytometric analysis was conducted using the BD FACSCanto II flow cytometer. A comprehensive panel of fluorochrome-conjugated monoclonal antibodies was used, targeting hematopoietic surface markers: CD34, CD117, CD13, CD33, CD41, CD61, CD45, HLA-DR, CD14, CD36, CD71, and others. Data were processed using FlowJo and BD FACSDiva software, and compared against established reference ranges.

Patients were also subjected to clinical evaluation, full blood count, peripheral smear analysis, and where indicated, molecular testing for JAK2 V617F, CALR, and MPL mutations. The correlation between immunophenotype and clinical/molecular findings was analyzed statistically.

Results:

Flow cytometric profiling revealed consistent differences among the MPN subtypes:

- Polycythemia vera (PV): Elevated granulocytic markers (CD13+, CD33+), absence of CD34+ blasts, and reduced expression of CD41. In some patients, the erythroid compartment was mildly expanded with a shift toward immature erythroid precursors.
- Essential thrombocythemia (ET): Increased expression of megakaryocytic markers CD41 and CD61, with preserved myeloid and erythroid differentiation. CD117 expression was variable, and CD34+ cells were minimal or absent.

- Primary myelofibrosis (PMF): Marked increase in CD34+ blasts, reduced HLA-DR expression, and high levels of CD71 and CD36, indicating ineffective erythropoiesis and aberrant myelopoiesis. Some patients showed an increase in double-positive populations suggestive of clonal heterogeneity.

Notably, flow cytometry was able to detect subclinical shifts in precursor populations, supporting early identification of clonal hematopoiesis prior to overt changes in peripheral blood counts.

Conclusion:

Immunophenotyping by flow cytometry is a valuable diagnostic tool in the assessment of MPNs. It offers rapid, reproducible, and quantitative analysis of hematopoietic cell populations, assisting in the discrimination of MPN subtypes, especially in cases with non-specific clinical presentations. Moreover, it provides insight into the clonal architecture and degree of dysregulation in hematopoiesis.

Routine application of this method, in conjunction with morphology and molecular diagnostics, enhances diagnostic precision, improves patient stratification, and may contribute to real-time monitoring of disease progression or treatment response. Future studies may refine antibody panels for even more targeted detection of early disease states or residual disease after therapy.

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