



IMMUNOFLUORESCENCE MICROSCOPY REVEALS PLATELET- AND RED BLOOD CELL ALTERATIONS AS MORPHOLOGICAL BIOMARKERS IN MYELOPROLIFERATIVE NEOPLASMS

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Myeloproliferative neoplasms (MPN) are clonal hematologic disorders characterized by heterogeneous clinical manifestations and variable prognosis, with thrombotic complications representing one of the most frequent and severe outcomes. Early diagnosis can be challenging, especially in patients presenting with nonspecific symptoms or isolated thrombotic events. In a previous work, we identified in a subset of MPN patients morphological abnormalities detectable by immunofluorescence microscopy on peripheral blood smears, namely reduced platelet alpha granule content and the presence of aggregates in red blood cells consisting of the cytoskeletal protein non-muscular myosin IIA (NMMIIA). The present study (DRKS-ID: DRKS00032588) aimed to validate these findings in an independent cohort and to assess the diagnostic performance of immunofluorescence microscopy in distinguishing MPN patients from healthy individuals based solely on morphological criteria. We enrolled 147 MPN patients and 99 healthy controls, age- and sex-matched. Standard peripheral blood smears were stained using primary and secondary antibodies and analysed by immunofluorescence microscopy. Two independent observers, blinded for subject identity and clinical information, evaluated all samples. Among the MPN patients, 65 (44%) had polycythemia

vera, 27 (18%) essential thrombocythemia, and 46 (32%) primary myelofibrosis. Red blood cell aggregates of NMMIIA were observed in 103 out of 147 patients (70%), co-occurring with reduced platelet alpha granule expression in 55% of cases. An additional 8% (12/147) of patients exhibited isolated alpha granule deficiency. In contrast, these alterations were uncommon among healthy controls: NMMIIA aggregates were detected in only 6% and alpha granule deficiency in 4%. Based on these findings, immunofluorescence microscopy demonstrated a sensitivity of 70.1% (95% CI: 62.7-77.5%) for identifying MPN through the presence of NMMIIA aggregates, and a specificity of 95.9% (95% CI: 88.2-98.7%) for detecting MPN through alpha granule deficiency. When both alterations co-occurred, specificity increased to 100%. These results confirm that the combination of NMMIIA aggregates in erythrocytes and diminished platelet alpha granule content represents a morphologic signature of MPN. Assessing these patterns by immunofluorescence microscopy can provide a useful, adjunctive diagnostic tool, supporting clinicians in identifying patients who warrant further evaluation for MPN—particularly in cases of unexplained thrombosis or subtle hematologic abnormalities.