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ELEVENTH ANNUAL MEETING

Diagnostic and Prognostic Utility of Flow Cytometric Enumeration of Aberrant hMICL–Expressing Circulating Leukemic Stem Cells in *BCR-ABL1*–Negative Myeloproliferative Neoplasms

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INTRODUCTION

- *BCR-ABL1*–negative myeloproliferative neoplasms (MPNs)—primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET)—lack specific discriminators an have overlapping features.
- Subcategorization is challenging, especially distinguishing ET from prefibrotic myelofibrosis.
- Human myeloid inhibitory C-type lectin-like receptor (hMICL; CD371) is a promising leukemic stem cell (LSC) marker that lack expression on CD34+CD38– cells in normal and regenerating bone marrow, making it a highly specific marker for LSCs and perhaps a crucial marker for subcategorization of MPNs.

AIMS

- To evaluate the utility of flowcytometric enumeration of circulating To evaluat the utility of flowcytometric enumeration of circulating CD34+CD38– hMICL+ cells for subcategorizing *BCR-ABL1*–negative MPNs.
- To correlate this analysis with the dynamic international prognostic scoring system (DIPSS) score.
- To determine the discriminatory cutoff for circulating CD34+CD38– hMICL+ cells that will identify PMF, including prefibrotic myelofibrosis, and in particula that will differentiate prefibrotic myelofibrosis from ET.

METHOD

Peripheral blood (PB) collected in EDTA- "54 patients and 20 controls"

Sample processing- standard stain-lyse-wash method (done within 4 hours of blood collection)

The following anti-human monoclonal antibodies (mAbs) used

Anti CD34 - PE CY7	Anti CD45-PERCP	Anti CD14 –APC CY7	Anti CD38 – APC	Anti hMICL-PE

Analysis - on BD FACS Canto II using BD FACSDiva™ v8.1.3 software.

The following cell subsets were analyzed using peripheral blood flow cytometry:

CD34+	CD34+CD38+ (committed progenitor cells),	CD34+CD38– (stem cells)	CD34+CD38– hMICL+ (LSCs)

In 54 BCR-ABL1–negative MPN patients (PV, 15; ET, 13; overt MF, 13; prefibrotic MF, 8; post-PV/ET MF, 5) who presented to the hematology department of a tertiary care center in North India over 18 months, bone marrow examination and mutational analysis were done.

RESULTS

- ✓ A Significant difference in the distribution of the CD34+CD38– hMICL+ subset among the MPN subgroups was demonstrated: The median in the overt MF, prefibrotic MF, and post-PV/ET MF groups was 2.8% , 4.15%, and 3%, significantly more than in the PV and ET groups (median, 0%; P<0.001).
- ✓ A discriminatory cutoff for the CD34+CD38– hMICL+ subset of 0.9 or greater predicted all myelofibrosis with a sensitivity and specificity of 88% and 89%. This also helped to differentiate prefibrotic myelofibrosis from ET.
- ✓ We found a significant correlation (ρ=0.41; P=0.036) between the proportion of CD34+CD38– hMICL+ cells and the DIPSS score. Every 1.72% increase in CD34+CD38– hMICL+ cells increased the DIPSS score by 1 unit.

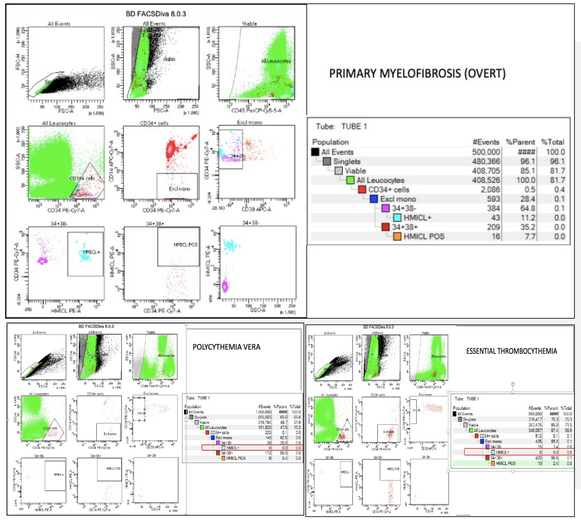
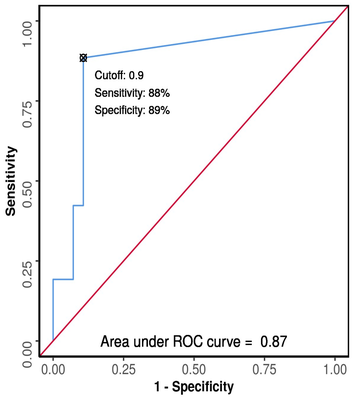


TABLE: Association between Diagnosis and Flowcytometry parameters

Parameters	Diagnosisaz					p value
	Polycythemia Vera (n = 15)	Essential Thrombocythemia (n = 13)	Overt MF (n = 13)	Cellular Phase of MF (n = 8)	Post-PV/ET MF (n = 5)	
CD34+Cells (%)***	0.82 ± 1.17	0.48 ± 0.64	3.28 ± 2.09	1.01 ± 0.86	1.10 ± 0.77	<0.001[1]
Proportion of CD34+CD38- Cells of CD34+Cells (%)	18.58 ± 21.95	24.89 ± 29.49	36.15 ± 38.35	30.99 ± 18.28	47.20 ± 22.67	0.127[1]
Proportion of CD34+CD38+ Cells of CD34+Cells (%)	81.42 ± 21.95	75.11 ± 29.49	63.85 ± 38.35	61.41 ± 28.92	37.54 ± 32.11	0.063[1]
Proportion of CD34+CD38- hMICL+ of CD34+CD38- Cells (%)***	1.51 ± 3.36	0.00 ± 0.00	5.72 ± 6.50	6.16 ± 6.78	2.42 ± 1.84	<0.001[1]

Figure : ROC Curve Analysis Showing Diagnostic Performance of Proportion of CD34+CD38-hMICL+ of CD34+CD38- Cells (%) in Predicting MF vs Non-MF (n = 54)



CONCLUSIONS

✓The precise diagnosis of MPN subtypes is crucial.

✓hMICL is a very specific leukemia stem cell marker.

✓Cut-off of Proportion of CD34+CD38-hMICL+ subset **≥0.9** predicted MF with **sensitivity of 88% and specificity of 89%.**

✓**C**ut off value used to distinguish MF from non-MF MPN i.e. ≥0.9% hMICL + cell. especiallymto distinguish MF (PMF and prefibrotic/early-MF) from ET as well.

✓CD34+CD38- hMICL+ subset holds a potential as a **diagnostic test especially for discriminating subtypes of MPNs**, tool for prognostication, an indicator of disease progression and a potential target for therapy.

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