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Study of *JAK2* V617F Allele Burden by Droplet Digital PCR in *BCR::ABL1* Negative Myeloproliferative Neoplasms and its Implications on Disease Phenotype

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INTRODUCTION

- Among the *BCR::ABL1* negative myeloproliferative neoplasms (MPN), the common ones, include, polycythaemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF); with the driver *JAK2*V617F mutation seen in 90-95% cases of PV, 50-60% cases of ET and 40-50% cases of PMF.
- The discovery of association of *JAK2*V617F mutation with *BCR::ABL1* negative MPN triggered a query on how a single mutation can cause different diseases with different clinical manifestations, and it was then reported that the *JAK2*V617F allele burden has association with the clinical phenotype of *BCR::ABL1* negative MPN[1].
- Allele burden is the ratio between the mutant allele and the wild type allele in a particular disorder caused by that mutation.
- The *JAK2*V617F allele burden has been found to correlate with the disease phenotype, presenting clinical features and disease outcome in *BCR::ABL1* negative MPN. Thereby, indicating that it is important to assess the *JAK2*V617F allele burden in MPN.

AIM OF STUDY

To study JAK2V617F allele burden in BCR::ABL1 negative MPN and correlate it with disease phenotypes and their clinical features, hematological parameters, prognostic scores and treatment response, using sensitive technique of Droplet digital PCR

MATERIAL AND METHODS

- All treatment naïve *BCR::ABL1* negative and *JAK2*V617F mutation positive MPN cases over period of 2 years were included in the study.
- Diagnosis of PV, ET, PMF and MPN-U was made as per the WHO criteria.
- Clinical details at presentation were noted, and symptoms were assessed as per the 'Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) or MPN TEN score'.
- Details for complete blood count, bone marrow morphology, *BCR::ABL1* fusion gene and *JAK2*V617F mutation were noted.
- *JAK2*V617F allele burden quantification was done on droplet digital PCR (ddPCR) (QX200, BioRad, USA), using - Primer Probe Mix: *JAK2*V617F Mutation Assay (Unique Assay Id- dHsam DW279446423/ dHsaMDM279446421; BioRad Laboratories, USA).
- Prognosis for PV and ET was assessed based on Prognostic risk score for thrombosis, and for PMF using IPSS and DIPSS scores.
- Treatment response assessment was recorded at 3 months and 6 months from the time of diagnosis. Primarily the following parameters were recorded to employ the above-mentioned criteria, such as: improvement in MPN-TEN score, hematological response, spleen response and development of thrombotic events.

RESULTS

- Eighty-six *BCR::ABL1* negative and *JAK2*V617F positive MPN cases were enrolled in the study and were categorized into polycythemia vera (PV) (n=39, 45.3%) essential thrombocythemia (ET) (n=12, 14%), primary myelofibrosis (PMF) (n=32, 37.2%) and MPN- unclassifiable (MPN-U) (n=3, 3.5%).
- Median age of patients was 56 years (range=22-81), with male:female ratio of 1.3:1.
- Statistically significant difference was observed between disease phenotypes and *JAK2*V617F allele burden (p-value=<0.001) (Table 1).
- PMF and PV cases exhibited the highest median allele burden of 74.7% and 74.3% respectively. ET cases had a lower median allele burden of 20.8%. MPN-U cases had the lowest median allele burden of 10.3% (Figure 1).

Table 1: Allele burden among the disease phenotypes

Disease phenotype	Allele burden (%) median (range)	p-value
PV (n=39)	74.3 (8.2 – 96.9)	<0.001
ET (n=12)	20.75 (11.9 –69.1)	
PMF (n=32)	74.7 (15.9 – 95.1)	
MPN-U (n=3)	10.3(8.6 – 29.4)	

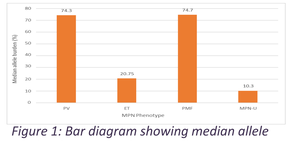

Figure 1: Bar diagram showing median allele burden among the MPN phenotypes

- PV cases showed a positive correlation of *JAK2*V617F allele burden with age, splenomegaly, total leucocyte count (TLC), IPSS-overall survival high-risk group and higher MPN-TEN score.
- In ET cases a positive correlation was found between *JAK2*V617F allele burden and mean platelet volume.
- In PMF cases, a positive correlation was found between *JAK2*V617F allele burden with TLC, platelet count and serum uric acid.
- No statistically significant correlation of allele burden was found with treatment response at 3 and 6 months in any group.
- Figures 2 to 5 show the peripheral blood smear, bone marrow aspirate smear, bone marrow biopsy section, reticulin stain section and ddPCR plots of one case each of PV, ET, PMF and MPN-U respectively.

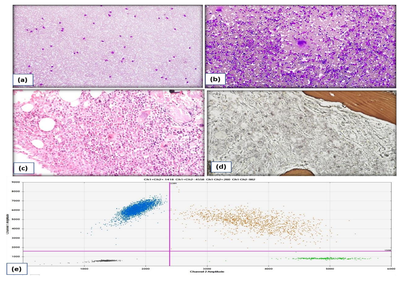

Figure 2: A case of PV: (a) Peripheral blood (PB) shows increased RBC density, (b) Bone marrow aspirate (BMA) shows panmyelosis (MGG x 200X), (c) Bone marrow biopsy (BMB) shows hypercellular marrow spaces [Hematoxylin & Eosin (H & E) x 400X], (d) Reticulin stain shows score of 0 (200X); (e) ddPCR two dimensional plot shows a large population of JAK2 V617F mutated cells with allele burden = 86.9%.

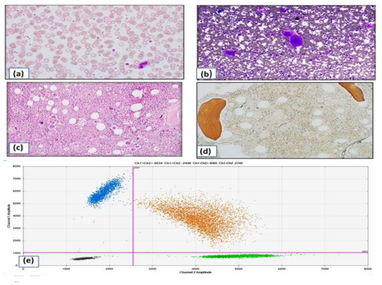

Figure 3: A case of ET: (a) PB shows thrombocytosis, (b) BMA showing large hyperlobulated megakaryocytes (MGG x 200X), (c) BMB shows megakaryocytic hyperplasia (H & E stain x 400X), (d) Reticul stain shows a score of 0 (200X), (e) ddPCR plots show JAK2 V617F mutated cells with allele burden = 42.1%, in a two-dimensional scatter plot

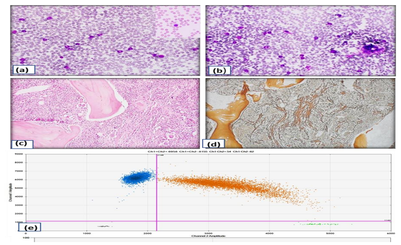

Figure 4: A case of PMF: (a) PB shows leucoerythroblastic picture, (b) BMA was aparticulate with few hematopoietic cells (MGG x 200X), (c) BMB shows fibrosis (H & E stain x 400X), (d) Reticulin stain shows increase in fibrosis with a score of 3 (200X); (e) JAK2V617F on ddPCR shows a large population of JAK2 V617F mutated cells with allele burden = 88.77%

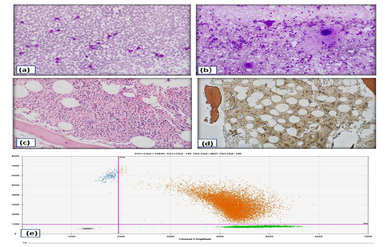

Figure 5: A case of MPN-U: (a) PB shows neutrophilia, (b) BMA is hypercellular and shows mild megakaryocytic hyperplasia (MGG x 200X), (c) BMB is mildly hypercellular (H & E stain x 400X), (d) Reticulin stain shows mild increase in fibrosis with a score of 1+ to 2+ (200X); (e) JAK2 V617F on ddPCR shows a JAK2 V617F mutated cells with allele burden = 29.4%.

DISCUSSION

- Table 2 summarizes details of studies done to correlate *JAK2*V617F allele burden with the disease phenotype in cases of *BCR::ABL1* negative MPN

Table 2: Studies correlating JAK2 V617F allele burden and the clinical phenotype of BCR::ABL1 negative MPN.

Authors	Country, year	Cases no.	Main findings
Zhou et al[2]	China, 2012	222	-JAK2V617F mutant burden was higher in PV =45.02 (35.1–54.2%) than in ET =28.2 (17.7–41.6%) patients. -Positive correlation was observed between allele burden and TLC and RBC counts in the PV group, whereas the ET group showed positive correlation with TLC and platelet counts.
Sook et al[3]	Korea, 2012	103	-Highest mean JAK2V617F allele burden was found in PV (66 ± 24.9%) followed by ET (40.5 ± 25.2%) and PMF patients (31.5 ± 37%). -No significant correlations were detected between allele burden with clinical or hematological parameters.
Yow et al[4]	Singapore, 2020	128	-Statistically significant difference in allele burden was seen, with ET patients having the lowest mean JAK2 V617F allele burden of 26.5% followed by PMF (44.0%), PV (48.8%) and MPN-U (76.4%).
Present study	India, 2026	86	- Statistically significant difference in allele burden was seen- with PMF and PV cases showing highest allele burden of 74.7% and 74.3% respectively. ET cases had lower median allele burden of 20.8%. MPN-U cases had the lowest median allele burden of 10.3%. -A positive correlation of JAK2V617F allele burden with TLC was seen in PV and PMF cases; and with MPV in ET cases. Also, age, splenomegaly, IPSS-overall survival high-risk group and higher MPN-TEN score correlated with higher allele burden in PV cases. -No statistically significant correlation of allele burden was found with treatment response at 3 and 6 months in any group.

CONCLUSIONS

- ***The JAK2V617F allele burden studied in different MPN groups, was found to be significantly different among them, with PMF and PV cases having the highest allele burden, and, ET and MPN-U cases having a lower allele burden.***
- ***JAK2V617F allele burden was also found to correlate with certain clinical and hematological parameters, in various MPN groups, more strongly in PV and PMF groups.***
- ***ddPCR for JAK2V617 allele burden used in study, was found to have high sensitivity with objective interpretation of results***

References:

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4. Yow KS, Liu X, Chai CN, et al. Asian Pac J Cancer Prev. 2020;21(9):2805–10.