ELSEVIER

Blood 142 (2023) 6396–6397

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

634.MYELOPROLIFERATIVE SYNDROMES: CLINICAL AND EPIDEMIOLOGICAL

Quantification of *JAK2* V167F in Patients with Myeloproliferative Neoplasms: Comparison of NGS and Digital PCR with Quantitative PCR

Ruth Stuckey, PhD[1], *Guillermo Santana*[1], *Yanira Florido*[1], *Adrián Segura-Díaz, MD*[1], *Maria Teresa Gómez-Casares, MD PhD*[2], *Cristina Bilbao-Sieyro, PhD*[3]

[1] Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas, Spain
[2] Hospital Universitario de Gran Canaria Dr. Negrín. Universidad de Las Palmas de Gran Canaria., Las Palmas de Gran Canaria, Spain
[3] Hospital Universitario de Gran Canaria Dr. Negrín. Universidad de Las Palmas. Morphology Department., Las Palmas, Spain

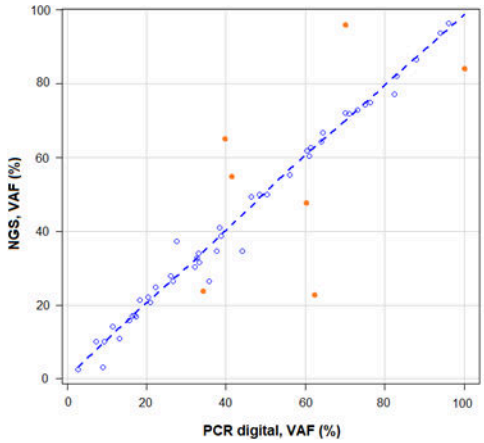
Introduction:
JAK2 V617F is the most common mutation in the Philadelphia-negative myeloproliferative neoplasms (MPN), detected in 97% of polycythemia vera (PV), and approximately 55% of essential thrombocythemia (ET) and primary myelofibrosis (PMF) cases. According to the WHO, *JAK2* V617F positivity is a major criteria for PV, ET or PMF diagnosis. But *JAK2* V617F quantification at diagnosis could be useful since a high allelic frequency (or VAF) has been associated with increased symptom burden in PV, risk of thrombotic events in ET and progression in PMF.
The current gold standard technique for *JAK2* V617F quantification is allele-specific quantitative PCR (qPCR). However, digital PCR (dPCR) is increasingly being employed since it permits absolute quantification of *JAK2* V617F without the need for a standard curve. QPCR *vs.* dPCR comparisons have shown high conformance between the two methods. Moreover, next-generation sequencing technologies (NGS) are increasingly used for MPN diagnosis.
The aim of this study was to evaluate the concordance between dPCR and NGS for *JAK2* V617F quantification at diagnosis.
Methods:
JAK2 V617F was quantified in the same sample of genomic DNA, extracted from total leukocytes from peripheral blood or bone marrow at diagnosis, by digital PCR (dPCR) and by NGS.
The Absolute Q™ Liquid Biopsy dPCR Assay (JAK2 12600) was used in the QuantStudio™ Absolute Q™ Digital PCR System (Applied Biosystems) with 25 ng DNA. This assay quantifies the number of *JAK2* WT-VIC and *JAK2* V617F-FAM positive signals in 20000 partitions, determined as copies *JAK2* V617F/µl input sample, and expressed as percentage VAF, with a limit of detection of 0.1%.
NGS was performed with the MiSeq (Illumina) platform and the targeted 30-gene panel Myeloid Solution™ (SOPHiA GENETICS) using 200 ng DNA. VAF was determined as the number of variant reads divided by the number of total reads (reported as a percentage), with a limit of detection of 2.5%.
Statistical analyses were performed using R-commander version 2.8. P-values <0.05 were considered statistically significant.
Results:
The quantification of *JAK2* V617F was carried out in a total of 52 patient samples with both techniques. Of the 52 patients, 5 had a diagnosis of ET and 47 had PV. The average VAF of *JAK2* V617F using dPCR was 14.1% and 48.2%, and 17.1% and 47.6% using NGS for ET patients and PV patients, respectively.
The concordance of positivity for *JAK2* V617F between the two methods was 100%.
With dPCR, the average VAF of *JAK2* V617F was 44.62% (range 2.7%-100%). With NGS, the average VAF of *JAK2* V617F was 44.95% (range 2.5%-96.5%), with an average coverage/read depth >3500 reads. At diagnosis, there was no difference in the mean VAF of *JAK2* V617F determined using dPCR *vs.* NGS (44.62 *vs.* 44.95, p=0.792, paired Student t-test). There was an excellent correlation between the VAF of *JAK2* V617F determined using dPCR *vs.* NGS (Pearson correlation coefficient 0.944, $p<0.001$; **Figure 1**).
Only 7 samples had a deviation >10% in the VAF quantification. These determinations are indicated in orange in the graph and listed in the table **.** The cause of 2/7 deviations might have been a limited DNA volume/concentration available for dPCR.

The high concordance of variant quantification by dPCR andNGS was maintained for *JAK2* V617F with low VAFs (≤20%, mean 12.0% *vs.* 11.8%, respectively; p=0.840) and high VAFs (≥80%, mean 90.6% *vs.* 89.9%, respectively; p=0.858).

Conclusions:

Overall, excellent correlation was observed between the VAF of *JAK2* V617F obtained using dPCR and NGS, showing that NGS is a robust quantification method. However, 7 samples had a difference >10% VAF, with a deviation >25% in 3 cases. Standardization of molecular techniques for *JAK2* V617F quantification is important if the VAF is to be monitored during MPN patient follow-up as a biomarker for disease evolution and for response to treatment, with VAF reductions shown for pegylated interferon and ruxolitinib. DPCR might be better suited for follow-up quantification due to its higher sensitivity and lower price, while NGS at diagnosis, apart from detecting and quantifying *JAK2* V617F at baseline, has the advantage of providing information on other gene mutations with prognostic value.

Disclosures Stuckey: *Gilead:* Other: travel expenses; *Novartis:* Other: travel expenses. **Segura-Díaz:** *Sanofi Genzyme:* Honoraria; *Novartis:* Honoraria. **Gómez-Casares:** *AstraZeneca:* Other: Training support; *AbbVie:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Astellas:* Research Funding; *GSK:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Novartis:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Pfizer:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Incyte:* Research Funding; *Janssen:* Other: Training support; *Gilead:* Other: Training support; *Amgen:* Research Funding. **Bilbao-Sieyro:** *Abbvie:* Honoraria.



	Digital PCR, VAF (%)	NGS, VAF (%)	Difference in VAF (%)	Reason for difference
1	62.2	22.8	39.4	Unknown
2	34.2	23.8	10.4	Unknown
3	60.3	47.7	12.6	Unknown
4	41.5	55.0	13.5	Unknown
5	39.7	65.1	25.4	Limited DNA
6	100	84.1	15.9	Limited DNA
7	70.0	96.0	26.0	Limited DNA

Figure 1

https://doi.org/10.1182/blood-2023-188510