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Detecting *JAK2* mutations in myeloproliferative neoplasms: can you please all the people all of the time?

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The well-known adage "You can please some of the people all of the time, you can please all of the people some of the time, but you cannot please all of the people all of the time" is often attributed to John Lydgate, an English poet and translator born in the 14th century, and was supposedly later paraphrased in an address by Abraham Lincoln, the 16th President of the United States of America. This phrase is readily applicable to cancer molecular diagnostics when considering the variables of assay sensitivity and specificity in the detection of somatically acquired, disease-specific mutations. A molecular diagnostic assay should not only aspire to detect all mutations intended but also at a clinically relevant level of detection. This challenge is pertinent for the molecular detection of myeloproliferative neoplasm (MPN)-associated *JAK2* mutations.

By far the most commonly acquired mutation in MPN is the p.V617F mutation (c.1849G>T) located in *JAK2* exon 14. It is present in more than 95% of patients with polycythemia vera and in more than half of those patients with essential thrombocythemia and primary myelofibrosis [1]. Identification of the *JAK2* V617F and other so-called "driver" mutations in *JAK2* exon 12, *MPL* exon 10 and *CALR* exon 9 is now an essential diagnostic criterion for MPN according to the World Health Organization classification of myeloid neoplasms [2]. Several methodologies exist to detect the *JAK2* V617F mutation and include allele-specific PCR, quantitative PCR (qPCR), melting curve analysis, high resolution melt analysis, restriction enzyme digestion, direct sequencing, pyrosequencing, denaturing HPLC, digital droplet PCR and next-generation sequencing (NGS) [3]. Each of these techniques has its own sensitivity and specificity with adoption of the required technique largely dependent of the clinical requirement with qPCR most widely utilised to date due to low cost and excellent sensitivity [4]. Diagnostic guidelines have indicated a sensitivity of detection of approximately 2% mutant alleles for routine diagnostic purposes [3,5,6] although it is widely acknowledged that *JAK2* V617F allele burdens less than this can result in an MPN phenotype [7]. Importantly, several other *JAK2* mutations have been described (in exon 14 and throughout the *JAK2* gene) in both sporadic and familial MPN, albeit at a low incidence, and it is such cases that may be underdiagnosed to lack of specificity of assays that only target the *JAK2* V617F [8,9].

The issue of sensitivity and specificity in the detection of *JAK2* mutations has recently been comprehensively addressed by Maslah, *et al.* [10]. Firstly in a head to head comparison, this group observed excellent concordance between their *JAK2* V617F qPCR and NGS approaches but only above the detection limit of their NGS assay which was 2% mutant alleles. However, due to the higher limit of detection of NGS, 25/473 (5.3%) samples were found to be discordant between the two techniques with the majority of these discrepancies were due to the failure of NGS to detect low *JAK2* V617F allele burdens less than 1%. Conversely, qPCR failed to detect the alternative *JAK2* exon 14 mutations of D620E, V615L and a different V617F resulting from a two nucleotide substitution. Secondly, this group used their NGS approach (all coding *JAK2* exons) to screen a large cohort of patients with suspected MPN and discovered 28 variants in 52/473 (11.0%) samples not including the V617F. Several of these variants were known single nucleotide polymorphisms (SNPs) or variants of unknown significance possessing allele burdens of approximately 50% most likely representing SNPs. Impressively, further somatic mutations (D751H, G281S, H345L, T817del) and variants previously described as germ line (E846D, N1108S, R1063H) were detected in 28/427 (6.6%) patients [10].

Maslah, *et al.* eloquently demonstrate the value of both qPCR and NGS approaches for the detection of the *JAK2* V617F and alternative *JAK2* mutations respectively. Clearly the advantage of qPCR is sensitivity at diagnosis and the capacity to quantitate low levels of measurable residual disease, especially relevant in the post-allogeneic stem cell transplant setting [11-13]. Despite this sensitivity, it is acknowledged that other MPN-associated *JAK2* mutations can occur and are present in exon 14 and throughout the coding region and these mutations result in false-negative qPCR results [14,15]. However, front-line sequencing the entire coding region of *JAK2* (and *MPL*) by NGS in all patients presenting with a thrombocytosis, an erythrocytosis, or any other MPN-related feature is probably unfeasible and unwarranted in the majority of diagnostic laboratories. Improvements are and will be made in the sensitivity of NGS approaches for detecting the *JAK2* V617F at lower allele burdens suggesting the future possibility of this approach for tracking measurable residual disease [16, 17]. Current knowledge therefore mandates NGS of *JAK2* (in clinically relevant cases) following *JAK2* V617F qPCR for an optimal diagnosis.

Competing interests

The author declares no competing interests.

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