CORRESPONDENCE

Repeat *JAK2* V617F testing in patients with suspected essential thrombocythaemia

Molecular investigation for characteristic initiating mutations, in addition to clinical, haematological and histopathological evidence, has become an integral part of myeloproliferative neoplasm (MPN) diagnosis. Detection of the *JAK2* V617F mutation and those within *CALR* exon 9, *MPL* exon 10 and *JAK2* exon 12 can be performed by a variety of methodologies each possessing its own characteristics of sensitivity, specificity and clinical applicability.[1] Mutation identification has been traditionally performed in a logical, stepwise fashion guided by other presenting features or increasingly in a simultaneous manner by next-generation sequencing (NGS). Up to 15% of patients with MPN of essential thrombocythaemia (ET) have no evidence of the canonical mutations (termed 'triple-negative') and are associated with a distinct clinical course particularly in regard to thrombotic risk therefore influencing treatment.[2] Low *JAK2* V617F allele burdens are by themselves insufficient to result in a diagnosis of an MPN in the absence of other diagnostic criteria.[3] Additionally, expansion of the *JAK2* V617F-positive haematopoietic clone(s) is highly variable among MPN patients and may occur over months or years.[4 5] It is therefore possible that a proportion of patients with a persistent thrombocytosis and suggestive clinical features may not be diagnosed as ET or alternatively, misdiagnosed as triple-negative ET if no canonical mutation is detected.

At this centre stepwise *JAK2* V617F screening is performed by non-quantitative allele-specific PCR, *CALR* screening by fragment length analysis with *MPL* exon 10 detection and *JAK2* exon 12 performed by NGS. The NGS assay also encompasses *JAK2* exon 14 to disclose possible rare variants in this exon.[6] Due to surplus testing and a finite NGS capacity, each stage is performed on request and not reflexively. The *JAK2* V617F allele-specific PCR and NGS assays have similar detection sensitivities of 2% allele burden. From the beginning of January 2019 to the end March 2020, 287 individual patient samples have been analysed by the NGS approach. In addition to the identification of *MPL* exon 10 in 5 patients (1.7%) and *JAK2* exon 12 mutations in three patients (1.0%), the NGS assay identified the *JAK2* V617F in repeat samples from four historical patients (1.4%) with a persistent thrombocytosis and suspected ET in whom this mutation was previously not detected by allele-specific PCR. These repeat samples were from 12, 24, 31 and 131 months after initial investigation with variant allele frequencies of 3.8%, 5.3%, 2.2% and 21.0%, respectively. All repeat samples had evidence of the *JAK2* V617F by allele-specific PCR. Bone marrow examination was performed in two patients at first presentation with megakaryocytic clustering and hyperlobation observed in both. All four patients had a bone marrow examination at or near the time of repeat molecular testing. The two repeat marrows demonstrated some further evidence of disease manifestation (increased megakaryocyte numbers and reticulin deposition, respectively). Of those two patients in whom bone marrow examination was performed at the time of repeat testing only, one patient did and one patient did not display definitive features of ET or an MPN.

The phenomenon of the *JAK2* V617F appearing in previously triple-negative ET has only been reported sporadically.[7 8] Here, the incidence in our routine, clinical, diagnostic practice is highlighted and shown to be at a similar level to *MPL* exon 10 and *JAK2* exon 12 detection rates. This concise review also affirms the continued value of bone marrow examination in MPN diagnosis in conjunction with molecular testing as distinctive morphological characteristics of ET were present in three of the four patients. It is acknowledged that a more sensitive mutation detection approach may have identified the *JAK2* V617F at first testing. It is, therefore, proposed that repeat *JAK2* V617F testing is performed in those patients with triple-negative thrombocytosis at least 12 months after first presentation (regardless of mutation detection technique) where bone marrow morphology and clinical features are evocative of an MPN. This would allow for potential clonal expansion into the detectable range, overcoming this pitfall and resolving the diagnosis prior to further investigations.

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