Review: JAK2V617F Allele Burden in Diagnosis and Therapeutic Monitoring of Myeloproliferative Neoplasms

Bhagya Dharmawickreme and Chaminidri Witharana

ABSTRACT

Characterized by overproduction of differentiated cells of myeloid lineage, polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative neoplasms (MPNs). Found in 95% of PV patients and 50-60% of ET and PMF patients, the JAK2V617F mutation is the most common molecular abnormality shared by the three MPN phenotypes. Although the JAK2 mutation is recommended for diagnosis of MPNs by the World Health Organization (WHO), its presence alone is insufficient to discriminate among the 3 subtypes. This implication of single mutation (JAK2V617F) in all three MPN phenotypes has long been an objective under question and several studies investigating on the gene dosage hypothesis have discovered the promising role of the JAK2V617F allele burden in MPN phenotype. The significant differences of the JAK2V617F allele burden in PV, ET and PMF patients as well as its associations with specific clinical and haematological characteristics bear high utility in diagnosis, prognosis, and therapeutic monitoring. Although great strides have been achieved with the use of qPCR and new molecular biology techniques in allele burden quantification, addressing the deficits in the current understandings and further improvement of technology will be highly beneficial. Therefore, we have reviewed PubMed database from 2005 to 2022.

Using keywords such as JAK2V617F mutation, Allele burden, Myeloproliferative neoplasms etc. and the present review discusses the significance of JAK2V617F allele burden in diagnosis and therapeutic monitoring of myeloproliferative neoplasms.

Keywords: Allele burden, cancer, JAK2V617F, myeloproliferative neoplasms.

I. INTRODUCTION

Myeloproliferative neoplasms (MPNs), a group of clonal malignant disorders characterized by overproduction of terminally differentiated cells of the myeloid lineage [1]. Specific driver mutations within hematopoietic stem cells are known to provide cytokine-independent proliferative signals leading to the overproduction of myeloid cells [1], [2]. The term myeloproliferative disorders, initially conceptualized by [3], underwent revision as myeloproliferative neoplasms, MPNs. According to the World Health Organization, MPNs include sub-categories with chronic myeloid leukemia (CML) and Philadelphia chromosome negative disorders such polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) [4], [5]. These sub-categories possess common driver mutations and overlapping clinico-pathologies, as well as the ability to evolve into other types and acute myeloid leukemia (AML), [6]. This adds to the complexity of the diagnosis, risk assessment and therapeutic choices of these disorders.

PV is considered to be the ultimate phenotype of Janus tyrosine kinase 2 (JAK2) gene mutations and is known to be the most common of all MPNs. The disease course is indolent, and lifespan is usually measured in decades. Characterized by erythrocytosis, with a progressive increase over time in erythrocytosis, granulopoiesis, and thrombopoiesis it is often associated with splenomegaly where isolated cases are also recorded. The most common complications being arterial and venous thrombosis, in some patient’s disease transformation to bone marrow failure, myelofibrosis and AML is recorded [6], [7].

Characterized by sustained thrombocytosis and increased megakaryocyte numbers ET is the most indolent of MPNs [6]. WHO 2016 guidelines for diagnosis of ET includes an elevated platelet count, the presence of characteristic bone marrow (BM) histology, the absence of defining features of any of the other MPNs or myeloid neoplasms, and the presence characteristic clonal genetic information [4], [8].

PMF is the least common and most aggressive of MPNs. Characterized by proliferation of abnormal megakaryocytes and granulocytes with a presentation of bone marrow fibrosis, PMF is accompanied with splenomegaly due to extramedullary haematopoiesis, an increase in circulating CD34+ cells and anaemia. The disease has a progressive course, leading to bone marrow failure, organ failure due to extramedullary haematopoiesis and transformation to AML [1], [6].
II. MPN PATHOGENESIS WITH DRIVER MUTATIONS

The pathogenesis of MPNs has been under scrutiny for years, until the identification of the driver mutations in more than 90% of patients with MPNs, which broadened the understanding of the disease [6], [9]. The driver mutations are associated with cytokine independent constitutive activation of signal transduction pathways responsible for haematopoesis [2]. Activation of the JAK-STAT pathway is a common denominator which has obtained prominence as a central theme in MPN pathogenesis [2]. A new era began with the discovery of JAK2 V617F mutation, which was followed by a series of additional descriptions of MPL and CALR mutations as MPN drivers. The driver mutations, found in JAK2, myeloproliferative leukemia virus oncogene (MPL) and calreticulin (CALR) genes, are often mutually exclusive and have unique disease kinetics that leads to clone expansion [1]. However other mutations in genes involving epigenetic regulation, RNA splicing, tumor suppressors and transcriptional regulators have been identified to cooperate with the drivers and play a key role in disease initiation, progression and leukemic transformation [1], [10]. Therefore virtually all MPN entities arise from a single mutated hematopoietic stem cell (HSC) that clonally expands and give rise to single or multilineage hyperplasia with respective phenotypes.[10]

III. JAK2 GENE MUTATIONS

A major breakthrough that provided substantial insight into the pathogenesis of MPNs was the discovery of JAK2V617F mutation in 2005 [11], [12]. Multiple groups following disparate approaches such as PV cell culture studies in growth factor deficient media which failed to proliferate in the presence of JAK2 siRNA [13] and study of Loss of heterozygosity resulting uniparental disomy (UPD) and identification of the JAK2V617F allele in the minimal region of UPD [14] together with other approaches led these different groups to identify the single most commonly mutated gene in MPN, which occurs in virtually all patients with PV and 50-60% of patients with ET or PMF [15]. The somatic gain of function mutation thus identified corresponds to guanine to thymidine transversion (G>T) at nucleotide 1849, in exon 14 of JAK2 resulting in valine to phenylalanine substitution at codon 617 (V617F) [10].

IV. JAK2

A member of the Janus kinase family located on chromosome 9 at locus p24, JAK2 serves as the cognate tyrosine kinase for the erythropoietin and thrombopoietin receptors and also for the granulocyte colony-stimulating factor receptor all of which lacks an intrinsic kinase domain [16]. In other words, JAK family kinases can be considered as catalytic parts of hematopoietic cytokine receptor family. Not only that the association of JAKs with receptors is identified to be important for their proper trafficking to the cell surface [1].

At the N-terminus of the JAK2 gene, there is a four-point-one ezrin radixin moesin (FERM)-like domain and a SH2 domain [10] and has a dual kinase structure comprising of a canonical tyrosine kinase domain (JH1), and a pseudokinase domain (JH2), which normally inhibits JH1 kinase activity in the absence of ligand binding. Though JH1-JH2 interactions are yet unresolved, the current understanding favors a trans association where JH2 pseudo kinase domain on one receptor monomer inhibits the JH1 kinase domain of the JAK2 molecule on its partner receptor monomer and vice versa [6].

Usually, in the receptors, cytokine binding induces changes in conformation which activates JAKs by transphosphorylation. Signaling via JAK2 activation causes phosphorylation of downstream signal transducers and activators of transcription (STAT) proteins, ultimately leading to cell growth and differentiation [10], [17].

V. THE JAK2V617F MUTATION

Corresponding to a nonsynonymous transversion of G>T in exon 14, the most common mutation JAK2V617F in JH2 pseudokinase domain is known to impair the physiologic inhibitory influence on JH1 kinase domain of JAK2 and cause constitutive kinase activation[10]. V617F mutation disrupts JH1/JH2 interactions through steric interference [18]. A probable mechanism that explains this effect involves changes in the JAK2 Src homology 2 (SH2)-JH2 linker region, which alters the interface between the JH2 and JH1 domains [6]. The constitutive activation results in cytokine hypersensitivity and ligand independent receptor activation. JAK2V617F induces constitutive activation of STATs as well as phosphatidylinositol 3-kinase (PI3K) and MAPK pathways. This results in an increased expression of mitotic proteins, and regulatory proteins of the cell cycle, cyclins D2 and CDC25A, which can disrupt the G1/S checkpoint and increase cell cycling [1]. [17]. In addition, several other downstream targets which can promulgate the malignant state in MPN when dysregulated include PU.1 and ID1 transcription factors which increase myeloid differentiation, anti-apoptotic genes such as B-cell lymphoma-extra-large (Bcl-XL) and Bcl2, which inhibit apoptosis and promote cytokine-independent growth [17]. Furthermore phosphorylation of PRMT5 by mutant JAK2, suppresses its arginine methyltransferase activity and leads to altered chromatin remodeling with increased erythroid colony formation and cell growth, phosphorylation of the Y41 residue of histone H3 by mutant JAK2, leads to displacement of HP1 protein from chromatin and increased gene transcription at loci of known protooncogenes, up-regulation of the La autoantigen by JAK2V617F, leads to impaired p53 activity are considered as several other downstream consequences of JAK2V617F that may engender MPN [18]. JAK2V617F mutation arises in a multipotent hematopoietic progenitor and is present in all myeloid lineages and can also be detected in lymphoid cells such as NK cells, B cells, and rarely, in T cells. It is absent in non-hematopoietic cells, although it has been found in endothelial cells of the spleen.

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and splanchic veins of patients with MF and/or splanchic thrombosis, such as in the Budd-Chiari syndrome [10]. The JAK2V617F mutation often undergoes a transition from heterozygosity to homozygosity due to the occurrence of mitotic recombination resulting in copy-neutral loss of heterozygosity [10]. Furthermore homozygosity for the JAK2V617F mutation associated with greater erythropoietin independence in hematopoietic progenitors is known to be seen in about one third of PV patients but rarely in ET patients [19].

VI. JAK2V617F ALLELE BURDEN

How a single mutation (JAK2V617F) form the basis of at least three major MPN phenotypes has long been a puzzle to the scientific community [19], [20]. Different hypotheses have been advocated, such as a different stem cell as the target of mutation, the genetic background [21], Pre JAK-molecular events and epigenetic factors [22], [23]. Variable level of JAK2 kinase activity as a reflection of the relative proportion of mutated and wild-type protein in the cell was one of them which gained much attention [19]. The concept of gene dosage postulates a counterpart between clinical manifestations and the proportion of JAK2V617F mutant alleles with the term ‘allele burden’ addressing the ratio of mutant and wild type JAK2 alleles in cells [24].

VII. JAK2 ALLELE BURDEN AND PHENOTYPE

Multiple studies have shown a substantial differences of allele burden in different MPN phenotypes [19], [25]. In the majority of cases, the lowest allele burden was recorded in ET and higher allele burden in PV [24], [25], with PMF patients recording varied results [27]. An allele burden greater than 50% was observed to increase the probability of an overt PV or myelofibrotic evolution [28]. This follows the idea that JAK2V617F mutation could influence the phenotype by altering the level of expression. For example, constitutive expression of JAK2V617F in knock in mice [29], [30] and murine haematopoietic cell models resulted in MPN like phenotypes, [31], with a rise in mutant JAK2 levels correlating with progression from PV like phenotype to myelofibrosis [31], [32]. JAK2V617F allele burden correlates with clinical and haematological characteristics. In addition to the significant difference of allele burden in MPN subtypes, a variety of studies conducted with large series of patients from different regions of the world, investigating the association of allele burden with clinical phenotype have showed that JAK2V617F allele burden correlates with specific clinical and haematological characteristics of MPN patients.[19], [33]. These include prognostic variables such as haemoglobin concentration, white blood cell (WBC) counts, platelet counts and spleen size [26], [34] For instance higher V617F allele burden in PV patients corresponds to a pronounced myeloproliferative phenotype with increased haemoglobin levels [35]. Studies have revealed a direct correlation of JAK2V617F allele burden with activation parameters favouring thrombosis and associations of JAK2V617F allele burden with thrombotic risk [36], [37]. However no significant correlation of JAK2 allele burden with thrombosis and risk of developing AML was reported in Italian PV patients [35]. In Chinese PV patients JAK2V617F allele burden had a significant influence on WBC counts [26]. In a cohort of Indian patients a higher JAK2 allele burden was recorded in patients with splenomegaly than patients without splenomegaly [34]. Hemorrhagic complications have been associated with higher allele burden in some studies while lower median allele burden in others [36], [38]. The use of allele burden in predicting cardiovascular complications in MPN has also been recorded [38], [39]. JAK2V617F mutation have shown variable associations with age, gender, disease duration and survival [35], [40].

VIII. JAK2V617F ALLELE BURDEN IN PV, ET AND PMF

In PV patient’s direct relationship between JAK2V617F allele burden and white blood cell (WBC) count, BM cellularity, Hb level and spleen size is recorded [34] with an inverse relationship with platelet count [35]. Also, increased allele burden has been often associated with aggravated symptoms and poor prognosis [37]. Studies have reported that male PV patients had a higher allele burden than females [44] while some did not find any significant correlation between Gender and allele burden [26], [34]. Thrombosis is a major cause of mortality in essential thrombocythemia and a higher JAK2 allele burden is associated with an increased risk of thrombosis [19], [45]. JAK2V617F allele burden has proven to be informative even in PMF [43]. Although the results from the mayo clinic varied, [46] statistically significant associations have been recorded with JAK2V617F load and haemoglobin levels and leukocyte count [43]. A lower allele burden was reported to be associated with shorter time to anaemia and shorter survival of PMF patients [43]. Consensus of the majority was on the association of higher allele burden with the increase of constitutional symptoms of PV and ET patients and of MF transformation [33].

IX. USE OF JAK2V617F ALLELE BURDEN FOR MEASURING RESPONSE TO TREATMENT

It has been found that JAK2V617F mutation could arise in clones harbouring other mutations [19]. Reduction of mutant allele burden however, does not necessarily mean the cure of disease, and multiple studies have reported convincing results indicating the utility of the JAK2V617F allele burden in measuring response to treatment. Allogeneic stem cell transplantation is the only potential curative therapy for

TABLE I: COMPARISON OF DIFFERENT STUDIES ON JAK2V617F ALLELE BURDEN AND CLINICAL PHENOTYPE

<table>
<thead>
<tr>
<th>No of patients</th>
<th>Technique</th>
<th>MPN type and allele burden</th>
<th>Ref</th>
<th>Findings correlated with allele burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>134</td>
<td>Real-time PCR</td>
<td>34.6, 25.8, 51.8</td>
<td>24</td>
<td>Age, WBC count</td>
</tr>
<tr>
<td>125</td>
<td>ARMS-PCR, qRT-PCR</td>
<td>72.4, 9</td>
<td>41</td>
<td>Leukocyte count</td>
</tr>
<tr>
<td>170</td>
<td>As-PCR, qRT-PCR</td>
<td>33.2, 7</td>
<td>26</td>
<td>Hb, WBC count, Splenomegaly</td>
</tr>
<tr>
<td>151</td>
<td>ARMS-PCR, qRT-PCR</td>
<td>56.8, 21.7, 56.1</td>
<td>25</td>
<td>No significant correlations</td>
</tr>
<tr>
<td>105</td>
<td>Pyrosequencing</td>
<td>46, *</td>
<td>42</td>
<td>Myelofibrosis, Splenomegaly</td>
</tr>
</tbody>
</table>
myelofibrosis yet burdened by high transplant related mortality. A follow up study 28 days after transplantation has shown that JAK2V617F allele burden >1% was associated with a higher risk of relapse and poor survival [47]. Others have also indicated its importance as a marker of minimal residual disease and in monitoring transplantation efficiency. Reduction of JAK2V617F allele burden in MPN patients was observed upon treatment with interferon (IFN-alpha) and JAK inhibitors. Similar decrease of allele burden was reported upon treatment with Hydroxyurea [41] while only few studies contradicted. Which indicates that the allele burden testing could be incorporated in the initial diagnostic work-up as an additional parameter to improve patient outcomes.

X. SIGNIFICANCE OF QUANTIFYING ALLELE BURDEN

Review of past studies has shown that the quantitative measurement of JAK2V617F allele burden is different in PV, ET and PMF, indicating the utility of allele burden as a marker for early diagnosis and differentiation of MPNs. The significant correlation of JAK2V617F allele burden with specific haematological and clinical characteristics, underlines its importance in risk assessment and patient stratification. JAK2 allele burden quantification has proven to be a valuable marker in drug response assessment and therapeutic monitoring. Overall, detection of JAK2V617F mutation status and allele burden is helpful in the differential diagnosis, prognosis, disease phenotype, complication, and evolution.

XI. QUANTIFICATION OF JAK2V617F ALLELE BURDEN

The starting point for the utilization of JAK2V617F allele burden in MPN assessment is accurate and reproducible quantification. Though DNA samples of peripheral blood or bone marrow are common, Studies with RNA has also shown promising results [48]. Many different techniques have been designed for JAK2V617F allele burden quantification of which quantitative real-time PCR remains a popular choice [49]. Considering the discrepancies of the results produced by different assays the method of choice should be specific and sensitive to detect low mutant allele levels [50]. A multicentre study that assessed concordance of assays designed for JAK2 allele burden quantification of different laboratories highlighted the importance of using well defined and accurate standards for calibration and suggested the use of plasmid DNA dilutions along with a well calibrated genomic DNA sample as an internal control for the most precise quantification [50]. In addition to allele specific PCR assays and Taqman assays, Next Generation Sequencing (NGS) has been shown to allow the detection of the V617F mutation with comparable performances, but weaker sensitivity to qPCR, with the advantage of detection of new potentially pathogenic JAK2 variants [51], [52]. Droplet digital PCR is a new addition to the existing technologies with the capability to achieve an absolute quantification without the need of a standard curve and ability to overcome some of the drawbacks of qPCR technique [28].

XII. CONCLUSION

More than a decade after the discovery of JAK2V617F mutation, with the genetic landscape of MPN much elucidated, revelation of the correlations of allele burden with Ph+ MPN phenotypes has given new insights to the understanding of the implication of a single mutation in multiple phenotypes. Identification of the differences of JAK2V617F allele burden in PV, ET and PMF could provide a means of early differentiation of MPN subtypes possibly before clinical manifestation. Significant associations of JAK2V617F allele burden with specific clinical and haematological characteristics are useful in prognostication, risk stratification and determining therapy. According to the literature JAK2V617F allele burden has predictive power in determining risk of thrombosis, evolution to MF and acute myeloid leukemia and even in the survival of MPN patients. Presence of an allele burden <1% has been suggestive of a lower risk of relapse after allogeneic stem cell transplantation indicating its importance as a marker for therapeutic monitoring. Although most of the studies dictate similar results on associations of allele burden and phenotype, few discrepancies could be attributed to the differences of the method used for allele burden quantification as well as sample sources. The number of the study population, demographic features as well as treatment modalities could also be causes of controversial results. The retrospective nature of most of the studies is identified as another limitation. Therefore, meta-analysis of the studies altogether and prospective studies are important, while improved technologies addressing limitations of current allele burden quantification methods will be highly beneficial. Also, similar studies using mutations other than JAK2V617F would be advantageous. Accordingly, it is clear that the JAK2V617F allele burden is a valuable marker that has high utility as a tool for diagnosis and therapeutic monitoring and its incorporation into diagnostic workflow would allow better management of MPN patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


