

# Identification of *JAK2V617F* Mutation on Myeloproliferative Disorders in Medan

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**Abstract**— Myeloproliferative disorders (MPD) form a range of clonal haematological malignant diseases, the main members of which are Polycythaemia Vera (PV), Essential Thrombocythaemia (ET), and Primary Myelofibrosis (PMF). The molecular pathogenesis of these disorders is unknown, but gene *JAK2*, which encodes a tyrosine kinase was found mutated in MPD. Identification of *JAK2V617F* mutation can facilitate doctors to diagnose and determine the therapeutic targets in patients with MPD. Study on this mutation is already much observed in developing countries, but in Indonesia, the examination of *JAK2V617F* mutation can only be done on a limited area, such as the *Eijkman Institute*, Jakarta and the *Laboratory of Biomolecular CEBIOR*, University of Diponegoro. The aim of this study is to identify of *JAK2V617F* mutation and to develop laboratory center particularly in TERPADU laboratory, University of Sumatera Utara as a method to diagnose MPD in Medan. We recruited patients from Haji Adam Malik, Pirngadi hospitals, private hospitals, and other haematology clinics from July until October 2016. The diagnoses of PV, ET and PMF were made according to the World Health Organization (WHO) criteria, based on peripheral blood counts and bone marrow histology. We obtained DNA samples and detecting of *JAK2V617F* mutation at TERPADU laboratory, University of Sumatera Utara. In this study, of MPD patients, the *JAK2V617F* mutation was observed in PV (58%), and ET (27%). Of 23 MPD patients, 10 patients was identified as positive *JAK2V617F* mutation.

**Keywords**— *JAK2V617F* mutation, myeloproliferative disorders, polycythaemia vera, essential thrombocythaemia, primary myelofibrosis

## I. INTRODUCTION

The Myeloproliferative Disorders (MPD) represent arrange of clonal haematological malignant diseases, with three main members: Polycythaemia Vera (PV), Essential Thrombocythaemia (ET), and Primary Myelofibrosis (PMF)[1]. These three disorders are all thought to reflect transformation of a multipotent haemopoietic stem cell, but their molecular pathogenesis remains obscure. The absence of

definitive diagnostic tests and the scarcity of randomised controlled trials make management of these diseases especially challenging [2], [3].

The myeloproliferative disorders are characterised by overactive haemopoiesis, with increased production of red cells and platelets the major feature of PV and ET, respectively. The main clinical complication in these two disorders is thrombosis, although haemorrhage can also happen. In the longer term, a few patients with PV and ET might develop myelofibrosis or acute myeloid leukaemia. Late-stage idiopathic myelofibrosis is characterised by bone marrow fibrosis, cytopenia, and splenomegaly and can also transform to acute myeloid leukaemia [4].

In the United States, the estimated prevalence of PV ranges from 44 to 57 cases per 100,000 people [5]. PV typically occurs in the 6th or 7th decade of life and occurs more commonly in men and in both men and women of East European Jewish ancestry [6,7]. The prevalence of ET is estimated to be 38 to 57 per 100,000 population and is the lowest among patient with Philadelphia chromosome-negative Myeloproliferative Neoplasma (MPNs). There may be a higher prevalence in younger women (approximately 2:1), and the median age at diagnosis is 60 years. The incidence of PMF in 2008 to 2010 was estimated to be 1 per 100,000 population. The median age at diagnosis is 67 years [5]. As noted earlier, patients with PV and other MPNs can develop myelofibrosis late in their disease course

*JAK2* gene provides instructions for making a protein that promotes the growth and division (proliferation) of cells. This protein is part of a

signaling pathway called the *JAK/STAT* pathway, which transmits chemical signals from outside the cell to the cell nucleus. *JAK2* protein is essential for controlling the production of blood cells from hematopoietic stem cells [8]. A somatic mutation, c.1887G>T (p.Val617Phe), ref. Sequence NM\_004972, commonly known as *JAK2V617F*, has been described in the majority of patients with PV and in a subset of patients with ET and PMF [4,9,10,11]. It is noteworthy that among the growth factors to which MPD hematopoietic progenitors are hypersensitive, EPO, SCF, GM-CSF, IL3, TPO and IGF-1 use *JAK2* for signaling [12]. It has been demonstrated that the valine-to-phenylalanine substitution at amino acid position 617 leads to constitutive tyrosine phosphorylation activity and promotes cytokine hypersensitivity [9], [10], [11]. Mutations in these genes confer constitutive activation of the *JAK-STAT* pathway and other pathways promoting differentiation and proliferation of different lineages [13]. The *JAK2V617F* mutation is present in approximately 95% of patients with PV, 58% with PMF and 50% with ET [14]. In *JAK2V617F*-negative PV cases, some mutations have been described in exon 12 of the *JAK2* gene, corresponding to approximately 3% of all PV cases. These mutations have not been described in PMF and ET [15].

Detection of *JAK2V617F* mutation is the major diagnostic criteria for MPD which is often used today. Detection of these mutations not only help in the diagnosis, but also help determine a therapeutic target in patients [16]. After detection of the *JAK2V617F* mutation, the diagnostic criteria for MPD no longer just refers to the clinical symptoms experienced by patients, but clinical studies against *JAK2* as targeted therapies were developed and have grown to anti *JAK2* reported at a meeting of the American Society of Hematology, one anti *JAK2* is used as a Tyrosine Kinase Inhibitor Erlotinib and Ruxolitinib [17].

The molecular diagnosis of *JAK2V617F* mutation is quite common in the developed countries. It could make assist the researchers, physicians and other health professionals to diagnose and determine the target therapy, and is expected to improve survival rate patient. Currently in Indonesia, examination of *JAK2V617F* mutation is still very limited area, such

as in *Eijkman Institute* and Laboratory of Center for Biomedical Research (CEBIOR) Medical Faculty, University of Diponegoro, Semarang. Study at CEBIOR Semarang, 50 patients with referral diagnosis of PV during May 2012 to April 2015, showed 30 patients (60%) were positive of *JAK2V617F* and incidence of PV increased every year [18].

The examination of *JAK2V617F* mutation has not been done in laboratories in Medan. This study aims to identify *JAK2V617F* mutation and to develop laboratory center particularly in TERPADU laboratory, University of Sumatera Utara, Medan as a method to diagnose MPD.

## II. METHODS

### A. Study Population

Peripheral blood samples were obtained from 12 patients with PV, 11 patients with ET and not found patients with PMF, at the Adam Malik, Pirngadi hospitals, private hospitals and other Haematology and oncology clinic in Medan, from July until October, 2016. The samples were referred to TERPADU laboratory of Medical Faculty, University of Sumatera Utara for *JAK2V617F* examination.

The diagnoses of PV, PMF and ET were made according to the World Health Organization (WHO) criteria, based on peripheral blood counts and bone marrow histology. Peripheral blood samples were used to detect the *JAK2V617F* mutation [4,9,10,11]. The study protocol was approved by the local Ethics Committee, and informed consent was obtained from all patients.

### B. Procedures

We took a blood sample from every patient. Peripheral blood cells were purified and DNA extracted.

### C. Mutation Screening

We designed primers to amplify coding exons and splice junctions of the gene. Primer sequences are available from the authors. Genomic DNA was amplified and PCR products were directly sequenced in both directions on ABI 3730 or 3100 machines by Big Dye terminator sequencing (Applied Biosystems, Foster City, CA, USA).

### D. Allele-specific PCR

80ng of patient's DNA was amplified in a 36-cycle PCR reaction at an annealing temperature of 58°C. We used 1mol/L of a common reverse primer and 0.5mol/L of two forward primers (panel). The first forward primer is specific for the mutant allele and contains an intentional mismatch at the third nucleotide from the 3'end to improve specificity (giving a 203-bp product); the second amplifies a 364-bp product from both mutant and wild-type alleles and serves as an internal PCR control. Genomic DNA was extracted using the Promega Wizard Genomic DNA Purification Kit. The presence of the *JAK2* V617F mutation was assessed as previously described [4] and using Go Taq® PCR Core System II by Promega. *JAK2* amplicons were obtained using primers, *JAK2* reverse: 5'CTGAATAGTCCTACAGTGTTCAGTT TCA3' , forward (specific): 5'AGCATTGTTTAAATTATGGAGTATATT 3' and forward (internal control): 5'ATCTATAGTCATGCTGAAAGTAGGAGAAA G3' [4].

### III. RESULT

In this study of 23 MPD patients, 10 patients were identified as *JAK2V617F* positive mutation (43%). The positive *JAK2V617F* mutation was observed in 7 of 12 PV patients (58,3%), and 3 of 11 ET patients (27,3%), but PMF patients were not found in this study.

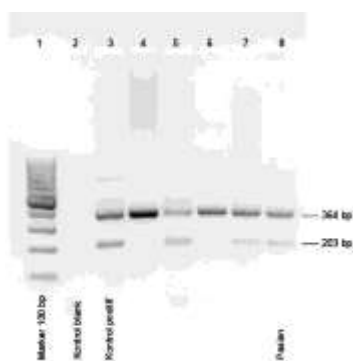


Fig.1 Number 1. Marker 100-bp, Number 2. negative control, Number 3. positive control, Number 4. *JAK2V617F* positive, Number 5,7,8. *JAK2V617F* positive showed two fragment 203-bp and 364-bp product.

In this study, the highest incidence of MPD showed in 8 patients with > 65 years old (34.8%) (see Table 1 below).

TABLE I  
GROUPING OF AGE OF MPD PATIENT

Group of age (years)	The number of MPD patient	
	Number of PV	Number of ET
< 25	-	3
25-34	1	2
35-44	2	-
45-54	-	1
55-64	3	3
>65	6	2
TOTAL	12	11

Sex related *JAK2V617F* mutation in PV patients showed the same in both sexes, but positive *JAK2V617F* was slightly more in women (33,3%) than men (25%). While for the ET patients positive *JAK2V617F* mutation was more in women than men. (Table 2)

TABLE II  
DISTRIBUTION OF PV AND ET PATIENTS RELATED SEX

<i>JAK2V617F</i> mutation	PV				ET			
	Men		Women		Men		Women	
	n	%	n	%	n	%	n	%
<i>JAK2V617F</i> positive	3	25	4	33,3	1	9,1	2	18,18
<i>JAK2V617F</i> negative	3	25	2	16,7	3	27,27	5	45,45
TOTAL	6	50	6	50	4	36,37	7	63,63

### IV. DISCUSSION

Of the 23 patients with MPD studied, the *JAK2V617F* mutation was detected in 10 patients (43%). The positive *JAK2V617F* mutation was observed in 7 of 12 PV patients (58%), and 3 of 11 ET patients (27%), but PMF patients were not found in this study. These frequencies are in agreement with those previously reported (Table 3)

TABLE III  
FREQUENCIES OF THE *JAK2V617F* MUTATION IN MEDAN PATIENTS AND IN PATIENTS FROM THE LITERATURE WITH MYELOPROLIFERATIVE DISORDERS

MPD	This study	Sihombi -ng et al (2015)	Santos et al. (2011)	Monte-Mor et al. (2007)	Baxter et al. (2005)	James et al. (2005)
PV	7/12 (58%)	30/50 (60%)	18/20 (90%)	47/49 (96%)	71/73 (97%)	40/45 (89%)
ET	3/11 (27%)	-	8/17 (47%)	8/29 (28%)	29/51 (57%)	9/21 (43%)
PMF	-	-	9/21 (43%)	14/25 (56%)	8/16 (50%)	3/7 (43%)

In this study, the majority of PV patients present with the *JAK2V617F* positive (58%), but

*JAK2V617F* negative were 42% patients. The majority of ET patients present with *JAK2V617F* negative. This shows the other mutations such as *JAK2* exon 12 mutations *MPL* and other unknown causes. Conversely, other mutations such as *TET2* or *IDH* mutations are occasionally seen in PV and ET [19,20,21]. Based on previous study, the *JAK2 V617F* mutation was detected in 18/20 (90%) cases of PV, 9/21 (42.9%) of PMF and 8/17 (47.1%) of ET [22].

Examination of *JAK2V617F* mutation, a new examination was first performed in TERPADU laboratory as a method to diagnose MPD in Medan. Number of MPD patients were collected during this study are as many as 23 patients. This amount was relatively small when compared to previous studies in CEBIOR (Semarang) which encountered by 50 patients with PV during the period of year 2012-2015. This is because the study period is quite short (4 months). But if it was seen by the incidence of this disease in the world, this may be acceptable because of its low incidence of MPD. The incidence of PV in the world was 0.02-2.8 per 100,000 per year; ET has an incidence of 0.1-1.5 per 100,000 per year and Myelofibrosis has an international incidence of 0.4-0.9 per 100,000 per year [23].

In this study the highest incidence of MPD occurred in > 65 years old as many as 8 patients (34.8%), where PV 6 patients (26%) and ET 2 patients (8.7%). It was relevant to study at laboratory CEBIOR, the highest prevalence rate occurred in the age 51-60 years was 23 patients (43.47%) and the smallest prevalence was <20 years old with 1 patient (2.17%) [18]. Previous study has showed that PV attacks range in ages of 50-70 years [23]. In the longer term, a few patients with PV and ET might develop Myelofibrosis or Acute Myeloid Leukaemia. Late-stage idiopathic myelofibrosis was characterised by bone marrow fibrosis, cytopenia, and splenomegaly and can also transform to acute myeloid leukaemia [4]. PV disease was most prevalent in old age, it can be explained that the increase of the allele was increased with age and the appearance of clinical symptoms and complications were not directly show after the onset of mutations, but mutations takes approximately 12 years to cause symptoms and complication [24].

Sex related *JAK2V617F* mutation in PV patients

occur the same in both sexes, but positive *JAK2V617F* was slightly more in women (33.3%) than men (25%) (Table 2). While for the ET patients, *JAK2V617F* positive was highly in women than men. It is relevant with previous studies in CEBIOR, that men (60%) more than women (40%) with PV, but *JAK2V617F* positive was mostly in women. The amount of allele was more burden in men than women. Although the mechanism of the influence of gender on *JAK2V617F* allele has yet to be clarified biologically, but there was speculation that less burden of alleles (homozygous) on *JAK2V617F* reflect the fewer mitotic recombination events that generate clonal expansion in *JAK2V617F* homozygote [25]. Therefore men suffering PV more than women. In this study, a mutation was more in women, may be caused the sample size was relatively small when compared to previous studies.

## V. CONCLUSIONS AND RECOMMENDATIONS

The *JAK2V617F* mutation examination conducted in TERPADU laboratory of Medical Faculty, University of Sumatera Utara on 23 patients with MPD in Medan period July to October 2016 and obtained *JAK2V617F* positive for 10 patients. This study is preliminary data for future studies with a longer period of time so that the number of patients who could accumulate more and development of TERPADU laboratory as center laboratory to *JAK2V617F* examination in Medan can be realized and helpful.

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